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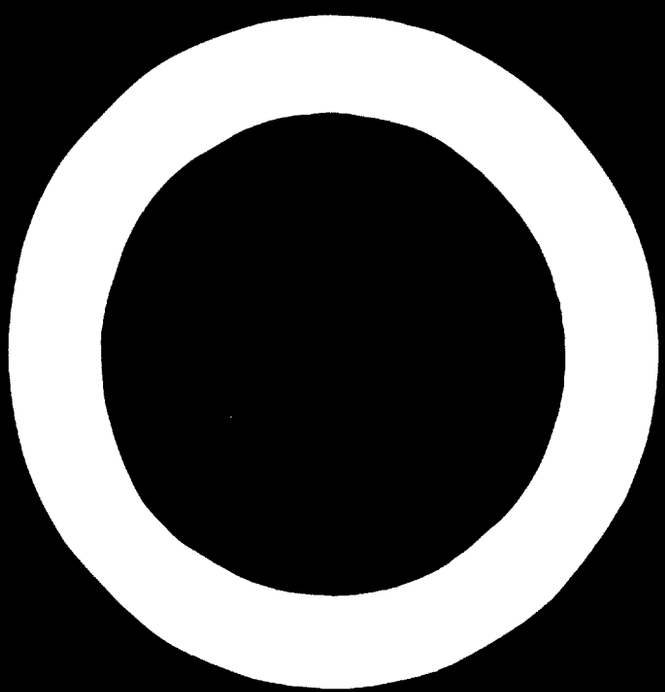
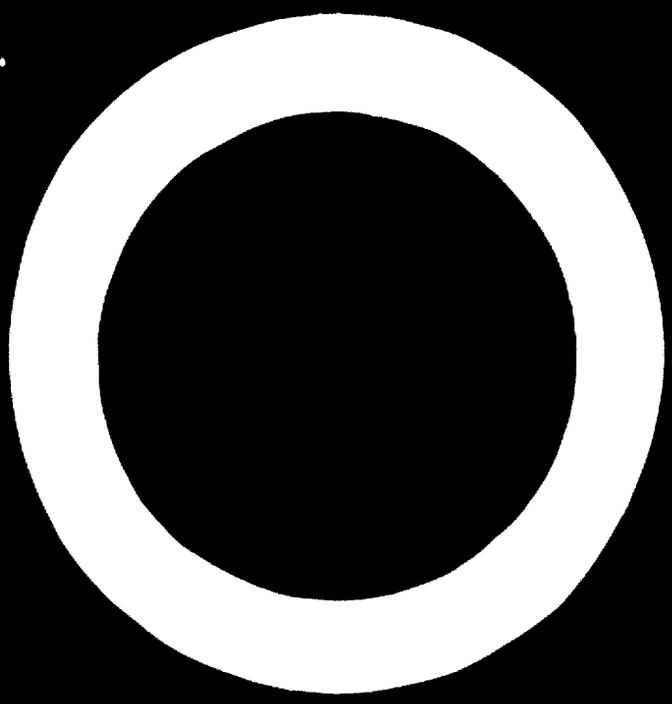
MANUFACTURE OF CHEMICALS BY FERMENTATION

Report and proceedings of an Expert Group Meeting held at Vienna from 1 to 5 December 1969

UNITED NATIONS





UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
VIENNA

MANUFACTURE OF CHEMICALS BY FERMENTATION

Report and proceedings of the Expert Group Meeting held in Vienna, 1–5 December 1969

UNITED NATIONS
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Contents

Contents (cont'd)

Explanatory note

Ton refers to metric ton (1,000 kg) unless otherwise indicated.

PART I REPORT

Introduction

1. The Expert Working Group Meeting on Manufacture of Chemicals by Fermentation was held in Vienna, Austria from 1 to 5 December 1969. Various aspects of the fermentation industry were discussed along with the presentation of papers on various specialized subjects in the field. Conclusions and recommendations were drawn up. The group visited a fermentation plant near Vienna during the meeting.

2. Fermentation is the earliest process of converting one substance into another and has been used for thousands of years; it has recently become an important branch of the chemical industry. In addition to the conventional products such as ethyl-alcohol, many new products can be produced by fermentation processes such as antibiotics, vitamins, steroid hormones, food ingredients, etc. Great potential exists in many developing countries where plenty of raw materials containing sugar and starch are available.

3. Taking the above into consideration the purpose of the meeting was, in general, to promote industrial development in the fermentation sector in the developing countries and, in particular, to examine the possibilities of the application of fermentation processes to the production of different products in developing countries.

4. The meeting was opened by Mr. Ibrahim Helmi Abdel-Rahman, Executive Director of UNIDO, who welcomed the participants on behalf of UNIDO and expressed the hope that this working group

would recommend concrete measures to be taken by the developing countries themselves, by UNIDO, and by other international organizations, such as the World Health Organization, the Food and Agriculture Organization and the International Atomic Energy Agency, to promote the development of the fermentation industry in developing countries.

5. UNIDO's introductory remarks were presented by Mr. C. S. Chiang, who gave a brief account of UNIDO's activities in industrial technology, especially in the field of fermentation industry and put forward a few ideas on some of the problems to be discussed in the meeting.

6. Twenty-four participants attended the meeting including ten experts (acting in their personal capacity and not as official representatives of their Governments), one expert from the World Health Organization, and thirteen observers from developed and developing countries. The participants are listed in annex 2. Professor Elmer Gaden (United States) was elected as Chairman, Dr. C. T. Calam (United Kingdom) as Vice-Chairman and Professor J. Meyrath (Austria) as Rapporteur of the meeting.

7. The agenda of the meeting is given in annex 1. Presentation of the expert papers was followed by discussion of the subject. Additional remarks were made by some of the observers during the sessions. After consideration of the papers and a general discussion, the participants joined in formulating technical recommendations and recommendations for specific UNIDO action to be taken in order to implement the conclusions arrived at during the meeting.

8. Part I of the present publication contains the report and conclusions of the group resulting from the meeting.

9. Part II of this publication contains the proceedings, consisting of: (a) summaries of papers presented to the meeting by experts from Austria, Canada, Federal Republic of Germany,

Hungary, Israel, United Kingdom and United States, and one by the representative of the World Health Organization; (b) a note of the talks given at the meeting by observers and by a representative of the International Atomic Energy Agency; and (c) a discussion of some aspects of manufacture of chemicals by fermentation.

10. The views expressed in the expert papers are those of the authors and do not necessarily represent the views of the secretariat of UNIDO.

General recommendations on the potential role of fermentation technology in developing countries

11. The Working Group outlined certain general circumstances under which fermentation technology is potentially valuable to developing countries. These are:

(a) When there is relatively little competition from processes other than fermentation (for example, petro-chemical process routes) and the products are needed in the local economy;

(b) When adequate supplies of raw materials (ordinarily carbohydrates) are available in the area;

(c) When the acquisition and practice of fermentation technology may provide an especially useful means for introducing industrialization into areas that are at a very low level of industrial development.

12. A combination of two or more of these factors may override purely technical or economic considerations and justify the local production of a specific chemical product by fermentation even when importation of this same product (perhaps produced synthetically) is less expensive.

13. The Group unanimously agreed, that separate responsibility in UNIDO for chemical production by fermentation and food production by fermentation (especially microbial protein) is not desirable. One group, or section, for both activities is preferable because essentially identical technologies are involved. In fact, differences between the manufacturing processes by fermentation for certain industrial chemicals may even be greater than those between microbial protein and many chemical fermentations.

Recommendations to UNIDO on procedures to be employed for evaluating the potential use of fermentation techniques in developing countries

14. The Group understands that UNIDO may receive requests from developing countries to review their potential use of fermentation techniques or may wish to suggest such reviews to specific

countries. In these situations, the Group recommends that UNIDO employ the following general procedures:

(1) Prepare a general report on the question, which should be circulated to appropriate agencies in developing countries, including industrial groups, research institutes etc. Special emphasis should be given to:

  (a) The types of products that can be manufactured by fermentation;

  (b) The kinds of raw materials that can be utilized, including waste or by-products from other technologies.

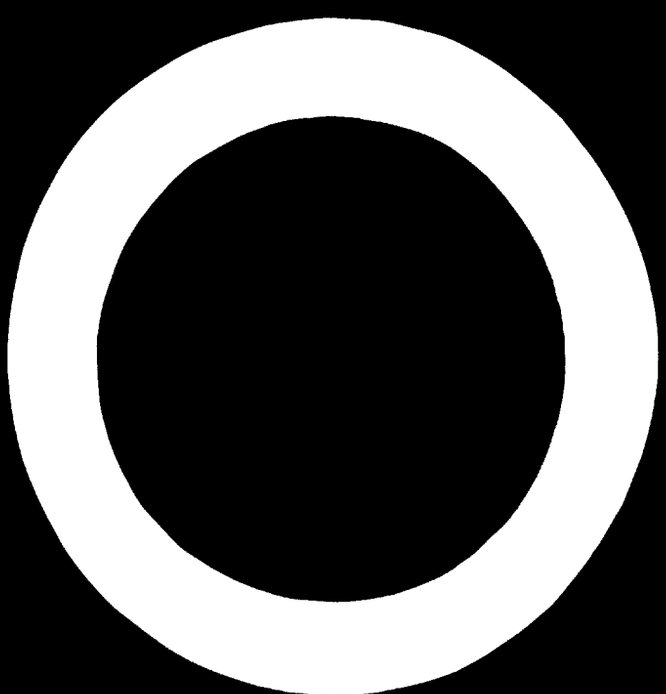
(2) Assemble a panel of experts to assist in evaluating inquiries and advising developing countries. This panel should be made up of individuals with broad experience in the practical aspects of fermentation, economic as well as technical. It is also highly advisable that this panel include representatives from those developing countries that have had experience with fermentation technology.

(3) When an inquiry is received from a particular country or area, make a preliminary review of the proposal in conjunction with the panel. The primary function of this preliminary study will be to guide the inquiring country in the preparation of a formal request and, especially, to provide background information, including data on local economic and technical conditions, which are necessary for a complete feasibility study.

(4) If this preliminary review indicates promise for the application of fermentation technology in the country, arrange a detailed feasibility study. To assist UNIDO staff or conduct such feasibility studies under contract, compile a list of government and industrial groups and individual consultants.

(5) Include in projects the appropriate measures to deal with the problem of training of personnel, adopting the methods of training suggested by the Group (see "Training", p.44).

(6) Investigate the establishment and maintenance of regional culture collections (see "Culture collections", p.45).



Annex 1

Agenda of the meeting

1. Organization and opening of the meeting and adoption of the agenda.

2. Lectures and discussions:

   Austrian fermentation industry and technology

   Fermentation and wastes disposal

   Biological treatment of wastes from antibiotic and related industries

   Industrial chemicals: organic solvents, organic acids, miscellaneous products, microbial insecticides

   Nutritional supplements: vitamins, amino acids and flavouring agents

   Fermentation processes employed in the pharmaceutical industries and their economic aspects

   Microbial production of therapeutic agents

   Energy and kinetic aspects of industrial fermentation

   Prospects for fermentation technology in developing countries

   Micro-organisms and their role in fermentation

   Use of radiation in the genetic improvement of industrial micro-organisms

   Problems of culture improvement in industrial microbiology

   Fermentation plant and equipment

   Antibiotic fermentations: problems in translation of laboratory and pilot-scale results to large-scale production

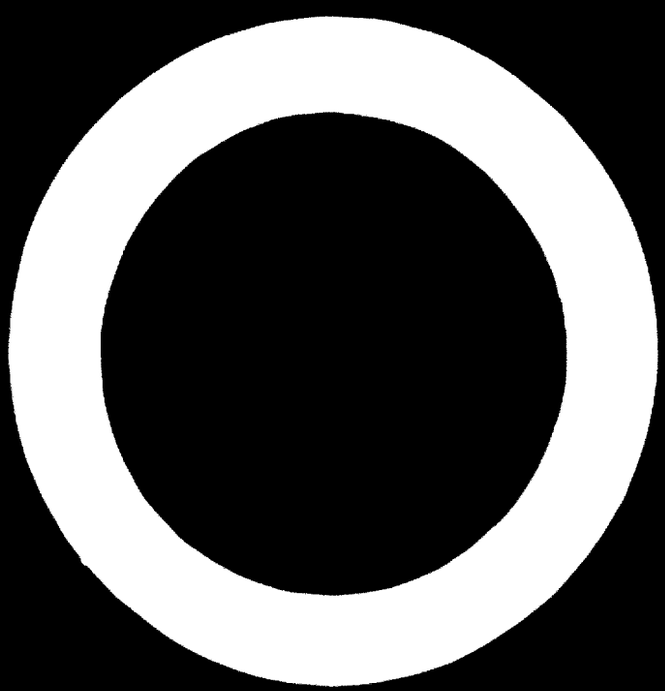
   Use of microbes in metal recovery from low-grade ores and sulphur production from gypsum

   Technological and training needs of developing countries

   Use of water-insoluble enzyme derivatives in synthesis and separation.

3. Visit to the fermentation plants of the Vereinigte Mautner Markhof'sche Presshefe Fabriken, Vienna.

4. Preparation and adoption of recommendations.



Annex 2

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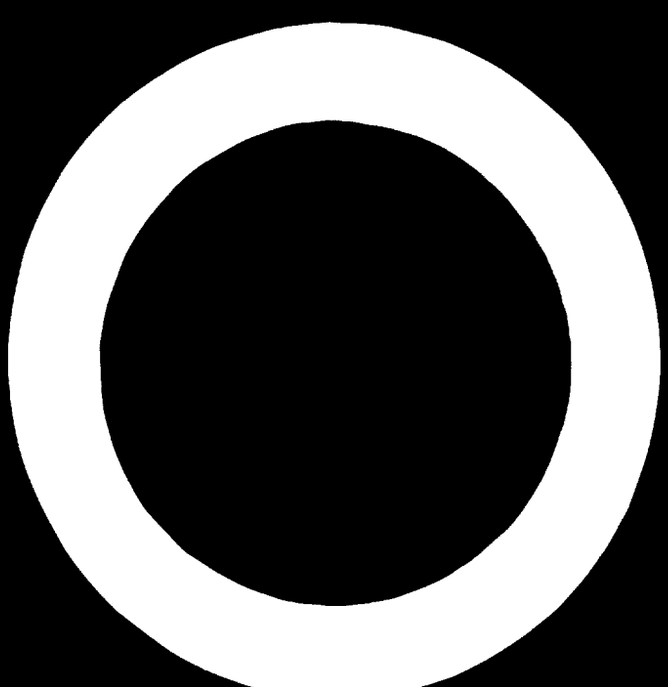
Annex 3

List of papers presented to the meeting[1/]

ID/WG.50/1 and Summary	Problems of culture improvement in industrial microbiology	C. T. Calam United Kingdom
ID/WG.50/2 and Summary	Metal recovery from low-grade ores, sulphur recovery from gypsum	J. V. Beck United States
ID/WG.50/3 and Summary	Nutritional supplements, vitamins, amino acids and flavouring agents	H. T. Huang United States
ID/WG.50/4 and Summary	Fermentation plants and equipment	E. L. Gaden, Jr. United States
ID/WG.50/5 and Summary	Use of water-insoluble enzyme derivatives in synthesis and separation	L. Goldstein Israel
ID/WG.50/6 and Summary	Micro-organisms and their role in fermentation	C. W. Hesseltine and W. C. Haynes United States
ID/WG.50/7 and Summary	Microbial production of therapeutic agents	C. Vézina Canada
ID/WG.50/8 and Summary	Fermentation processes employed in the pharmaceutical industries and their economic aspects	I. Horváth Hungary
ID/WG.50/9 and Summary	Fermentation and wastes disposal	P. A. Stevens WHO
ID/WG.50/10, Add.1 and Summary	Energetic and kinetic aspects of industrial fermentation	J. Meyrath Austria
ID/WG.50/13	Industrial chemicals: organic solvents; organic acids, miscellaneous products; microbial insecticides	F. Wagner Federal Republic of Germany

1/ A limited number of copies are available upon request.



PART II PROCEEDINGS

SUMMARIES OF PAPERS PRESENTED

Use of microbes in metal recovery from low-grade ores and sulphur production from gypsum

by Jay V. Beck

This paper summarizes the processes in which microbes effect the production of metals and sulphur. The role of microbes in the mineral industry is not generally known and appreciated. The paper is put forward therefore as a preliminary source of information; its fairly complete and up-to-date bibliography should be of value.

In developed countries the mining industry is well aware of the possibilities of using microbes in mining operations, but this may not be true of developing countries. The attention of national agencies, mining bureaux and - owing to the applicability of the process on a small scale - small local mining interests engaged in metal recovery from sulphide ores should be drawn to the subject matter of this paper. The reworking of disused mines or mill dumps offers a promising source of metals, which may be recovered at small capital cost.

The use of Thiobacillus ferro-oxidans in copper and uranium mining: Copper recovery from low-grade sulphide ores

The mining and recovery of copper from low-grade ores by leaching accounts for a significant part of total copper production. Studies have shown that the bacterium Thiobacillus ferrooxidans, which rapidly oxidizes and solubilizes sulphide ores, plays a major role in leaching operations on raw material that would otherwise never be used. The temperature and oxygen content of gases in the

interior of some ore dumps are incompatible with microbial activity, but it is suggested that bacterial oxidation and solubilization are important in low-temperature leaching and even at high temperatures. By adjusting leaching conditions to foster bacterial growth, this method of treating low-grade ores can be made much more efficient. Further improvements in efficiency may be anticipated when more is known about the bacterium and its action in leaching systems.

Uranium production from uranium oxide/iron pyrite ores

Bacterial leaching by using Thiobacillus ferrooxidans in uranium mine water is of some economic importance, but steps to improve the process have not met with sufficient success to justify its use for general uranium production. Recently, a reverse-flow, six-tank continuous process has been proposed for the rapid extraction of uranium from its oxide ores by bacterial action, which may prove to be economically competitive with the conventional method of extracting uranium, by acid oxidation.

The major economies obtainable by bacterial leaching lie in the reduced quantity of acid required and the lower temperature requirements compared with acid leaching - 25 lb instead of between 60 and 80 lb of sulphuric acid per ton of ore processed. The reason for this difference is that bacterial leaching generates considerable quantities of acid by oxidation of the pyrites. A temperature of 70°C is essential for chemical leaching, whereas bacterial leaching takes place at ambient temperatures.

The use of bacteria in sulphur production

Attempts have been made to use sulphate-reducing bacteria for commercial production of sulphur. The most successful of these was carried out in the United Kingdom and successfully reached the pilot plant stage with a daily production of about 200 lb of sulphur. The project was discontinued about ten years ago but is now being revived.

An interesting and significant aspect of the British project was the use of raw sewage from London as the source of the bacterial agent. It was hoped that the process would prove capable of removing much of the soluble organic matter from domestic sewage in addition to producing valuable sulphur from an inexpensive raw material, namely gypsum.

Problems of culture improvement in industrial microbiology - methods of improving industrial strains

by C. T. Calam

The methods used for mutation, isolation and testing of mutants are summarized in this paper. Examples are given of the use of different mutagens, including radiations and chemicals. The theory and practice of screening are discussed and reference is made to automated methods.

The object of the paper is to describe practical methods of working. The general procedure involves: (a) mutation, (b) isolation of about 200 cultures, (c) testing of these cultures in single flasks, (d) retesting of the best 50 cultures, using 3 to 4 flasks and (e) remutation of the best five cultures and repetition of the cycle. Taking the five best cultures of remutation has a number of advantages over taking the single best strain.

In discussing techniques, the importance of using reliable, simple methods that give reproducible results is stressed. Success depends on reliability and number of isolates screened. The importance of noticing and seizing opportunities and of forestalling difficulties is stressed. Patience and persistence are needed as progress may become slow after a time.

It is important that the first screening tests be carried out under conditions that as closely as possible resemble industrial processes to be used.

Following subjection to the mutagenic agents the organisms (about 200) are plated out and tested in single shaken flasks.

The best 50 strains are then selected, and further tests are carried out in triplicate or quadruplicate, keeping the best five strains. This cycle is then repeated. With a good team one cycle can be carried out every six weeks.

Experience has shown that it is far better to isolate and keep the cultures showing moderate improvement and subject them to remutation, instead of trying to find very significant improvements after the first mutation programme.

Automatic procedures or combinations of handwork and automated processes can be used successfully.

A section of the paper is devoted to hybridization. The reasons this method has given rather disappointing results are discussed on a theoretical basis. The comparatively poor success of hybridization techniques is due to the necessity of a large number of genes being involved in improving the fermentation for a particular product, and the high degree of improbability of finding homozygous cultures in the F_2 generation. Often a limited advance in productivity occurs, after which further progress becomes very difficult or impossible.

Methods, apart from the sexual process, have included the para-sexual process, transformation and transduction.

Success in both mutation and hybridization depends on the availability of good strains, some strains mutating more readily than others. The possibility that UNIDO could help in obtaining strains is suggested. (See also below: Micro-organisms and their role in fermentation, C. W. Hesseltine and W. C. Haynes.)

Fermentation plant and equipment

by E. L. Gaden, Jr.

Fermentation technology appears to be about to undergo its first substantial expansion in many years. The prospects for large-scale production of microbial protein are excellent and the manufacture of enzyme products is already accelerating. We should

therefore be able to look forward to the construction of a number of new fermentation plants and to increases in the capacity of many old ones. If these expectations come to pass, engineers responsible for design of the new facilities will face a variety of intriguing problems for which past experience and current practice offer little guidance.

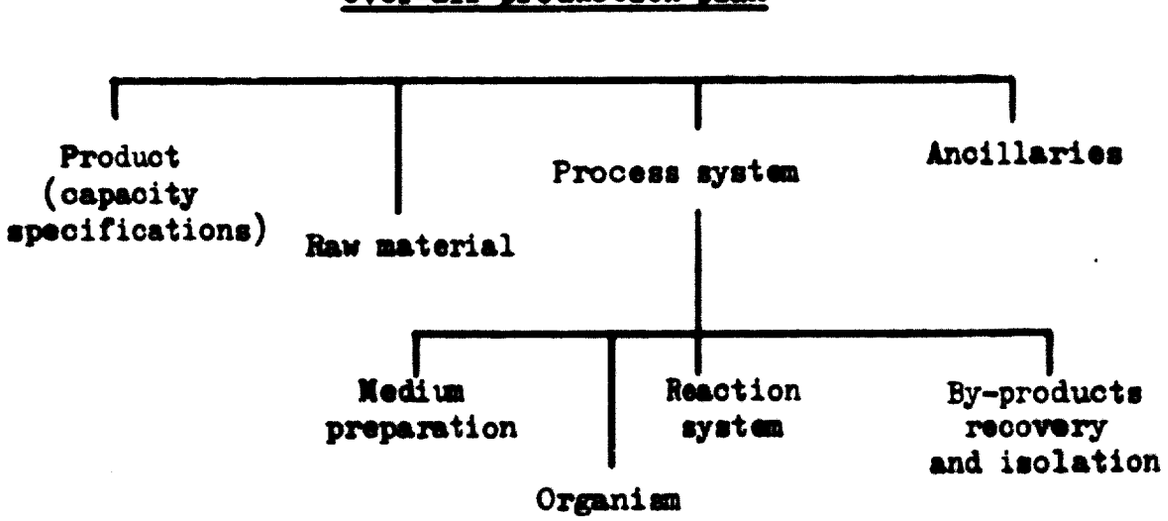
The diversity that we see in products of fermentation technology tends to conceal the uniformity that pervades the design of equipment and plant for producing these materials. In terms of current facilities there are only two types of fermentation processes. The first comprises biomass production by the propagation of cells in a highly aerated medium under conditions that might be called aseptic - or perhaps simply clean. Yeast manufacture dominates this class, and is almost the only one of significance at present. Biomass production seems certain to comprise a substantially greater fraction of the total output by fermentation methods in the years ahead.

Second, and most significant in economic value, is the formation of metabolites by the cells as they grow. Examples are: citric acid, amino acids, growth substances, enzymes and antibiotics.

The third class of microbial processes is more difficult to define. It includes those in which the cells are used to bring about a limited and often simple chemical change in the substrate. Microbial transformation of steroids is an excellent example, but the production of gluconic acid by the oxidation of glucose is an equally valid one.

The various process systems, i.e. batch, continuous and intermediate, are briefly discussed in the paper. A number of critical points with regard to reactor systems for microbiological processes are elucidated and discussed. It is stressed that all processes should be suited to local needs.

Establishment of a plant requires organization at several levels, as indicated below:



It is stressed that even in chemicals manufacture very few so-called continuous processes are truly continuous. With regard to some newer developments, certain drum-type fermenters are shown to be particularly attractive, the larger sizes of drum being equipped with internal agitators instead of themselves rotating.

In deep tanks, sonic nozzles give good oxygen-transfer rates but poor agitation. Air-lift fermenters have been shown to be of interest for microbial protein production in mashes with low viscosity. It is stressed that the plant should be kept flexible in all respects. One important aspect is not to choose fermenters that are too large; while the minimum size of fermenters for most processes may be about 40 m^3, the maximum size should not greatly exceed 100 m^3 for aerobic fermenters.

Regarding the material to be used for fermenters, there is usually no reason for choosing stainless steel. For corrosive mashes, newer plastic-type materials are attractive, such as F.R.P. (fibre glass). There may also be a future for concrete fermentation equipment.

In aseptic operations, the inoculation technique has often been faulty in the past; the aeration system has not so often been at fault. It is important also not to employ bottom valves.

Contamination occurs frequently during starting up, when there is condensation water in many parts of the system, including the filters. Mist eliminators are important and, as an insurance, membrane filters with pre-filters can be inserted at the entrance to the fermenter.

In the ensuing discussion Dr. Hesseltine reported that while he agreed with Dr. Gaden, for the most part there was still a use for solid substrate fermentations in the production of certain chemicals, e.g. certain enzymes (see also: The use of bran for production of spores, Vézina). Regarding the necessity, as reported by Dr. Iyengar, for stainless steel in penicillin fermenters, the Chairman pointed out that the results of the experiments reported may reflect the particular conditions, especially their scale.

Use of water-insoluble enzyme derivatives in synthesis and separation

by L. Goldstein

Water-insoluble enzyme derivatives have been prepared by:

(a) Adsorption on inert carriers or ion-exchange resins;

(b) Entrapping in gel lattices;

(c) Covalent binding to insoluble polymeric carriers; and

(d) Covalent crosslinking using bifunctional reagents.

The kinetic behaviour of immobilized enzyme systems is dominated by several factors not encountered in the kinetics of free enzymes:

(a) Effects of the chemical nature of the carrier, stemming from the modified environment within which the immobilized enzyme is located;

(b) Steric restrictions imposed by the carrier; and

(c) Diffusional control on the rate of substrate penetration.

Anomalies in the pH of the solution and in the Michaelis constants of many polyelectrolyte enzyme derivatives can be related to the unequal distribution of the various kinds of ions between the charged solid phase of enzyme particles and the surrounding solution. Proteases bound to polyelectrolyte carriers have been shown to exhibit restricted specificity towards protein substrates.

The flow-rate at which substrate perfuses through an enzyme column has been shown to affect the degree of conversion of substrate to product, the apparent Michaelis constant, and the apparent rate constant. Anomalies between the pH of the solution and the activity of papain-collodion membranes can be explained by the generation of pH gradients locally within the membrane, as a result of the liberation of hydrogen ions during hydrolysis of an ester substrate.

Kinetic analysis has shown that under stationary state conditions, a membrane-bound enzyme oannot attain its maximum activity, except when acting on a poor substrate. Immobilized enzymes have been used to attain better regulation of enzymic processes and for the preparation of enzyme electrodes. Enzyme columns have been utilized in automated analytical prooesses and in affinity chromatography for single-step purification of specific inhibitors. Reversal of the latter prooess has been employed for the purification of enzymes. Large-scale oolumns, operated continuously on an industrial scale have been shown to be superior to the batch processes utilizing non-adsorbed enzymes.

Micro-organisms and their role in fermentation

by C. W. Hesseltine and W. C. Haynes

This paper emphasizes the use of micro-organisms in fermentation in the developing countries. Inasmuch as the key to success or failure in most fermentation processes is availability of the proper micro-organisms, the characteristics of suitable microbial strains are enumerated; the industrial microbial oollections of the world, their locations, their general holdings, and the names of their directors are listed. The attributes of a good culture oollection are described.

The sources of new strains of micro-organisms for fermentation are the isolation of new wild strains in nature and from strains held in culture. Various fermentation processes in use

throughout the world are listed, together with the specific micro-organisms used to carry them out. The processes most likely to be useful in developing countries are indicated. The means are discussed by which small fermentation plants may acquire suitable microbial strains as are also the problems of maintaining stable cultures. For those interested in the isolation of their own cultures, a workable and proven scheme of operation is presented. Considerable space is devoted to the question of shipment of micro-organisms through international channels and also to legal problems relating to patents involving micro-organisms.

In the opinion of the authors it is unrealistic to maintain culture collections in developing countries. A number of considerations for culture collections are discussed.

The importance of collaboration of culture collections with IAMS (International Association of Microbiological Societies) is stressed.

Fermentation processes employed in the pharmaceutical industries and their economic aspects

by I. Horváth

The discovery of penicillin, the elaboration of its mass production, and its introduction into therapy marked the emergence of a new branch of research and a new method of production in the pharmaceutical industry, which as a result entered a phase of extremely rapid development. Today, 25 to 30 per cent of pharmaceutical products are manufactured by this technology; among other products, the most important antibiotics, vitamin B_{12}, ketosteroids, and enzymes are manufactured by sterile fermentation processes. The advance of technology and the isolation of biologically active, high-yielding strains reduced the initial prime costs. This was demonstrated by the price levels that developed after the patents had expired and has ensured that the production of most antibiotics by chemical synthesis will not become economical even in the long-term future. The various fermentation processes used in the

pharmaceutical industry are summarized in a table which gives the price, the probable cost of production and the patent situation.

The principles for the establishment of an industrial fermentation plant in a developing country are discussed and consideration is given to the production of important antibiotics, first of all penicillin, then tetracyclines and vitamin B_{12}. At current market prices, the smallest economic production unit is estimated to be of a fermentation capacity of 200 tons of working volume.

In a separate chapter a brief summary is presented of the major manufacturing processes, including an account of the requirements for a processing division in a generally flexible fermentation plant. In connexion with the more important processes the main trends of development are surveyed. The chapter deals with the following products and developments:

(a) Penicillin G and penicillin V.

(b) Tetracycline group - oxytetracycline and tetracycline. The therapeutic importance of new tetracycline derivatives.

(c) Basic antibiotics - streptomycin, neomycin. Pharmaceutical importance of substances isolated on the basis of similar technological principles.

(d) Macrolide antibiotics - erythromycin, oleandomycin.

(e) Antifungal antibiotics - nystatin, candicidin, griseofulvin.

(f) Technological developments in the production of vitamin B_{12}.

(g) Technology of the production of enzymes.

(h) Utilization of by-products.

Informatory economic data are given about fermentation plants for the most important products (penicillin, streptomycin, tetracyclines, vitamin B_{12} etc.) on the scale of 200 tons annual production capacity. A brief summary is given of the economic data for other fermentation products.

Nutritional supplements, vitamins, amino acids and flavouring agents

by H. T. Huang

The introductory part of this paper reviews the significance and uses of nutritional supplements, i.e. vitamins, amino acids and flavouring agents, in human and animal nutrition. The main part of the paper deals with specific supplements which are produced commercially by fermentation, i.e. riboflavin, vitamin B_{12}, glutamic acid, and lysine.

Technology of production for each product is discussed in detail in terms of culture selection, fermentation process, mechanism of biosynthesis, process control, product recovery and economics.

Finally, fermentation processes for other nutritional supplements of interest are described. These include the vitamins - carotene and ascorbic acid; the essential amino acids - threonine, trytophan, isoleucine and valine; and miscellaneous flavour products such as mushroom mycelia, oriental fermented foods and 5'-nucleotides.

Riboflavin can be produced by Eremothecium ashbyii and Ashbya gossypii, the latter being the more important today. Being plant pathogenes care has to be taken to avoid their dissemination during production of riboflavin. Substrates, usually consisting of ground lentils and pancreatic digests of gelatine, are used. Glycine content is a critical factor. The price of riboflavin in the United States in 1953 was $100 to $130 per kg. The yield by fermentation was about 2.5 g/l at that time; currently, yields of more than 10 g/l are reached. The total production in the United States is about 300,000 kg per annum, with a total value of about $7 million.

Monosodium glutamate can be produced by special strains of Bacillus megaterium-cereus. Production of this flavouring agent is now worldwide while in 1963 there was hardly any outside Japan. Yields of 60 to 80 g/l are reached, based on 60 per cent conversion of sugar. Biotin content of the medium is very critical in

order to obtain high yields. Recovery can be accomplished by acidification to precipitate glutamic acid. Production costs, which were about $70/lb in 1953, are today about $0.45/lb.

Lysine can be produced by mutants of glutamate-producing cultures with a yield of 56 g/l in 80 hours of fermentation time. In Japan alone 3,000 tons were produced in 1969, at a price of $1.95/lb, which appears a rather inflated price (Prof. Wagner pointed out that lysine could be produced synthetically at $0.25/kg.) Biotin content of the medium is critical in order to prevent an accumulation of glutamate.

Beta-carotene is not yet produced on an industrial scale, but the process seems to be ripe for industry to take up.

5'-nucleotides are important flavouring ingredients and can be used in 1/50 to 1/10 of the amount of monosodium glutamate. They can be obtained from nucleic acids by ion exchange chromatography. Mixtures (1:1 monosodium glutamate and 5'-nucleotides) are sold in the United States today at $9.60/lb, and it is very likely that the price will be further reduced.

In the discussion, in response to a question about methionin, Dr. Huang pointed out that there is very stiff competition between the above process and synthesis, since optically inactive mixtures produced by synthesis can be used to upgrade food and feedstuffs.

Energy and kinetic aspects of industrial fermentation

by J. Meyrath

The basic aspects of the kinetics of growth and fermentation of microbial cultures are briefly reviewed. Several proposals are discussed for characterizing mathematically the various phases of culture development. Certain essential factors are discussed which affect the various phases of growth and fermentation. The special problems are examined in some detail of fungal cultures, in which the initial phases of culture development influence the properties

displayed at later stages. The importance of self-stimulating and self-inhibiting substances is revealed.

Some fundamental questions are reviewed regarding the use and results of continuous cultivation of micro-organisms, in partioular the factors determining the choice of single-stage or multi-stage operation.

Energy aspeots of yeast development under aerobic and anaerobic oonditions are examined, as well as the more recent process of continuous alcoholic fermentation with extremely high rate and little surplus yeast produotion. In this process the yeast requires virtually no more than maintenance energy to keep up its activity. Besides energy considerations of biomass production from carbohydrates under aerobic conditions, the utilization of strongly reduced carbonaceous compounds (i.e. hydrooarbon) is examined in detail. Explanations are proposed for the rather poor energy utilization of this energy source.

Microbial production of therapeutic agents

by C. Vézina

A number of points with regard to the production of currently available antibiotios are outlined. Product improvement by fermentation technology, by microbial genetios and by environment are discussed in some detail.

In antibiotics production the price of the medium is not as critical as it is in some other fermentations, e.g. citric acid. In antibiotics production there is usually a rapid growth phase followed by an accumulation phase. Very often the extraction procedure is the more expensive part of the process, as is the case in penicillin production.

The search for new antibiotics is justified in view of the development of resistence to existing products among sens'tive pathogenic micro-organisms. It is emphasized that in selecting new strains the primary screening process should be cheap.

Detailed considerations for the production of penicillin are presented, and the importance of such points as strain selection, fermentation media, fermentation process and recovery is elucidated.

In the transformation of steroids, the use and re-use of spores (conidia, e.g. Aspergillus ochraceus) at high densities is explained in some detail. Spores are often produced on bran cultures. Processes are also described for produoing conidia by submerged growth in as short periods as 36 hours. Conidia remain active for several years and can also be transported to other factories, to be used there without further manipulation, provided they are kept in a frozen state.

The production of other microbial metabolites of therapeutic value, such as alkaloids, polysaccharides, and enzymes is briefly described.

Industrial chemicals: organic solvents, organic acids, microbial insecticides and miscellaneous products

by F. Wagner

Organic solvents

Ethanol

Since industrial ethanol can be produced much more cheaply by chemical synthesis (using ethylene and natural gas as raw materials), this fermentation process loses ground steadily.

Regarding fermentation techniques, it may be mentioned that vessels of 250 m^3 can be used with sugar concentrations of 15 to 20 per cent. Continuous processes on an industrial scale have been used with starchy raw materials as well as with molasses. Contamination, which may prove troublesome in continuous fermentations, is counteracted by acidification, or by additions of penicillin or sodium pentachlorophenolate.

Butanol and acetone

Competition from chemical synthesis is likewise extremely severe in the case of butanol and ethanol. In the fermentation process, 60 per cent of the production costs are accounted for by comparatively expensive raw materials (mainly molasses) and 15 to 20 per cent by fuel and power.

With regard to the process itself, contamination is very troublesome, particularly by bacteriophage. The sugar concentration is usually not higher than 7 per cent with conversion coefficients for solvents of up to 33 per cent. The continuous process has been proved on a laboratory scale.

2-butanediol and 3-butanediol

The organisms potentially useful for this fermentation are Aerobacter aerogenes and Bacillus polymyxa. Citrus press juice, citrus molasses, blackstrap molasses, beet molasses and sulphite waste liquor are possible raw materials.

A. aerogenes can ferment higher concentrations of sugar than B. polymyxa, while the latter has the advantage of possessing diastatic activity.

The major technical problems involved are the recovery of the products, due to their very high boiling point (180° to 184°C). Counter-current extraction methods are possible.

Polyhydric alcohols

Erythritol, arabitol and mannitol in addition to glycerol can be produced by osmophilic yeasts, where up to 0.6 grams of polyols can be formed per gram of glycose utilized. There is a chance that production of glycerol by osmophilic yeasts rather than by the old sulphite fermentation process may prove economically feasible, but so far very little glycerol is prepared by fermentation. Small amounts can easily be obtained by saponification of fats; large-scale production by synthetic means is based on the use of allylchloride, acrolein and propylene oxide.

Organic acids

Citric acid

Annual world production (estimated at 200 to 220 million lb) is predominantly by fermentation. A very little is extracted from fruit.

Concentrations of up to 25 per cent sugar are fermented, usually in shallow trays with *Aspergillus niger*, requiring incubation periods of 7 to 12 days. Yields range from 70 to 90 per cent based on glucose. The raw materials are molasses, fully hydrolysed starch of various origins, or sucrose.

There are numerous uses of citric acid, mainly in the food industry in various ways as an acidulant.

Itaconic acid

Aspergillus terreus and *A. itaconicus* are usually used either in surface or submerged fermentation, with cane molasses, raw sugar or cane juice as major raw material. Conversion coefficients of 60 per cent can be obtained, and continuous process has been shown to be possible.

Itaconic acid has some application in the manufacture of certain types of synthetic resins and detergents.

Gluconic acid

Microbial production of gluconic acid is an important and expanding industry. Annual world production is estimated at 30 to 40 million lb, but in view of the fact that gluconic acid is now being successfully applied in the concrete industry, there may be a rapid rise in production. Submerged processes with *Aspergillus niger* are comparatively easy; glucose concentrations of 38 to 45 per cent are used with aeration rates of 1.0 to 1.5 volumes of air per volume of mash per minute. Fermenters with "contactor agitation" give conversion yields of 95 to 97 per cent with aeration rates of 0.2 to 0.4 volumes of air per volume of mash per minute and fermentation times of 36 to 40 hours.

Microbial insecticides

Bacteria pathogenic in insects are currently being produced and used in practice. Bacillus thuringiensis, the causative agent of fatal diseases in many lepidopterous insects, is produced by conventional fermentation. Bacillus popilliae, pathogenic in the Japanese beetle is propagated only in the larvae of this insect.

Miscellaneous products

Dihydroxy acetone

There are some uses of this product in the pharmaceutical industry (as a sun-tanning agent). The raw material is usually glycerol, and the conversion agent is one of various members of the genus Acetobacter.

Sorbose, fructose

Sorbose is used quite extensively in the synthesis of ascorbic acid, and is produced by selected strains of Acetobacter on sorbitol in concentrations of 20 to 30 per cent. Nearly complete conversion of the raw material can take place in 30°C in 45 hours.

Mannitol can be almost quantitatively converted to fructose with selected strains of Acetobacter, and the final concentration of fructose can be as high as 35 per cent. Another possibility of fructose produotion is by fermenting glucose to gluconic acid and having fruotose intact. There are important medical applications of fructose (parenteral injeotions) and it is used also as a sweetening agent.

Polysaccharides

Dextran is ourrently produced by strains of Leuconostoo on sucrose with fructose as by-product. In order to produce dextrans with a higher degree of standardization of molecule chain-length, enzyme conversion with the isolated dextran sucrase can be carried out.

Xanthan gum, which is of value in the pharmaceutical, cosmetic, food and textile industries, oan be produoed with Xanthomonas campestris, using glucose, suorose or other carbohydrate sources as raw material.

Fermentation and wastes disposal

by P. A. Stevens

Among the residues of human life and activity are many fermentable substances and the wastes of fermentation industries. Wasted substances are naturally assimilated into the environment by various processes, including fermentation. Natural assimilation is limited, and when the volume of wastes exceeds these limits, nuisance and health hazards invariably result.

Protection of the public health requires as a minimum that wastes be safely confined and disposed of, and in some cases that they be suitably treated to prevent pollution of the environment. The wastes of the fermentation industries are in general amenable to biological treatment. Economic and social considerations lead to efforts to recover usable materials or to prevent the generation of wastes. Waste management in developing countries involves planning simple, adequate facilities, geared to present and possibly changing future requirements. Industries must bear a fair share of the responsibility and cost of waste treatment and disposal.

The World Health Organization has been actively assisting its Member States to carry out relevant studies and to build up competent staff and institutions. An International Reference Centre for Wastes Disposal was set up in 1968.

NOTES ON TALKS GIVEN AT THE MEETING

The Austrian fermentation industry

One of the participants, J. Meyrath, gave a review of the fermentation industry in Austria. He traced its development, particularly in recent years, and explained its present structure.

Biological treatment of wastes from antibiotic and related industries

An observer, M. R. S. Iyengar, described the oxidation ditch system for the biological treatment of wastes arising in industries manufacturing antibiotics and related products. He presented details of the processes that have shown promising results.

Use of radiation in the genetic improvement of industrial micro-organisms

The genetic composition of the micro-organisms used in industrial fermentation processes can be improved by irradiation. The work being done in this field by the International Atomic Energy Agency was briefly described by R. Mukherjee of the Radiation Biology Section of the Agency.

Antibiotic fermentations: problems in translating laboratory and pilot-scale results into large-scale practice

M. R. S. Iyengar also spoke briefly on another aspect of the manufacture of antibiotics by fermentation: the translation of results obtained in laboratory and pilot-scale experiments to full-scale commercial operations. He discussed the problems that can arise in regard to cultures and to the interrelationships between cultures and the physical systems employed.

The role of industrial fermentation in Cuba

An observer, E. D. Posada, talked about Cuba's experience of industrial fermentation. Of the 3 million tons of molasses produced in Cuba, the greater portion is used as animal feed (together with urea, for cattle). The remainder is used for

protein biosynthesis and for alcohol production. In view of the intensively developing use of molasses for animal feed, molasses is no longer abundantly available for fermentation processes and efforts are being made to use many kinds of waste materials in its place. Molasses is currently replacing 30 to 50 per cent of the carbohydrate portion of cattle fodder and efforts are being made at the same time to increase the protein content of molasses by the addition of soya beans. The importance was also mentioned of pelletizing essential amino acids, for example, in the rations for cattle feeding. This enables the rations to pass through both stomachs of ruminants.

SOME ASPECTS OF MANUFACTURE OF CHEMICALS BY FERMENTATION IN DEVELOPING COUNTRIES

In the course of the discussions at the meeting, the Group brought up some aspects of the manufacture of chemicals by fermentation that deserve consideration in the development of the fermentation industry in developing countries.

Techno-economic considerations in the manufacture of the principal fermentation products

(1) Organic solvents and other neutral compounds

Ethanol

Ethanol for industrial purposes can be produced more cheaply by chemical synthesis than by fermentation if it is produced on a large scale. UNIDO will advise each country concerned whether it can meet better the local demand and potential exports currently and for the coming ten to fifteen years by fermentation or by chemical synthesis. If marketing and export organization are sufficiently good, and if the necessary capital is available, a chemical plant will be advocated.

Raw materials: Molasses, cereals, potatoes, cassava, camote, sulphite, waste liquor.

By-products: Carbon dioxide (e.g. for soft drink manufacture), fusel oil (solvent).

Technology: Anaerobic fermentation; starchy substrates require hydrolysis; amylase production plant necessary for amylo process but saccharification is carried out with a growing mould (Rhizopus) rather than an amylase preparation.

Waste products: Slops.

Experience required: Little.

Plant flexibility: Plant usually not multi-purpose; unsuitable for production of butanol and acetone, which require highly aseptic working conditions.

Butanol and acetone

General remarks as for ethanol.

Raw materials: As for ethanol.

By-products: Carbon dioxide, hydrogen and riboflavin.

Technology: As for ethanol, but reliable sterilization and prevention of contamination absolutely necessary.

Waste products: Slops.

Experience required: Considerable micro-biological experience, particularly for manufacturing control; qualified workers in the plant to carry out sterilization, inoculations and transfers.

Plant flexibility: Relatively low; fermentation part suitable for anaerobic processes only.

(2) Organic acids

Acetic acid

While acetic acid can be made on an industrial scale more cheaply by synthesis, a small demand can be met by fermentation.

Raw materials: As for ethanol, including distilled ethanol itself, adjusted to about 14 per cent concentration, supplemented with nutrients.

By-products: None.

Technology: Preferably submerged fermentation, rather than percolating process. Alcoholic mashes from yeast fermentation can be fermented directly if an extraction procedure is used for acetic acid.

Experience required: Reliable personnel to control fermenters.

Plant flexibility: Relatively low, unless stirrers and agitators are specifically adapted; percolating plant not usable for other processes.

Lactic acid

General remarks as for acetic acid.

Raw materials: As for ethanol, plus whey; preferably pure raw materials such as glucose are to be used.

By-products: Lactic acid bacteria cultures.

Technology: Fermentation process comparatively easy; recovery costs high; stainless steel or plastic equipment required in recovery stage.

Experience required: Relatively little for fermentation stage; chemical engineering ability for recovery stage.

Plant flexibility: Low; fermentation plant can be used for ethanol fermentation but scales of production are wholly different.

Citric acid

The amount of citric acid that can be obtained by extraction from citrus fruits does not usually cover the requirement of citric acid in any country. Manufacture by synthesis is unsuitable for citric acid.

Raw materials: Molasses, sucrose, starchy materials.

By-products: Mycelium (as fertilizer).

Technology: Submerged process and surface fermentation both possible; extremely difficult technology; process unreliable unless all factors under very careful control; surface fermentation less subject to variation, but in any case citric acid fermentation is the most hazardous of all fermentations; use of starchy materials requires enzymic saccharification with α-amylase plus amylo-glucosidase.

Experience required: Extremely high level, both in the fermentation and in the recovery stage.

Gluconic acid

Gluconic acid is usually used as a means to supply Ca^{++} or Fe^{+++} to humans and animals; in future it is likely to be used as an additive to concrete, to improve its hardening qualities.

Raw materials: Glucose.

By-products: Glucose oxidase (oxygen-removing agent), mycelium as fertiliser.

Technology: Submerged aerobic process; glucose production from starchy material requires enzymic saccharification with α-amylase and amylo-glucosidase.

Experience required: Reliable workers to maintain cultures, prepare inocula, carry out sterilization, inoculations and transfers.

Plant flexibility: Good; suitable for other aerobic fermentations.

(3) Antibiotics

Penicillin G and penicillin V

Penicillins are still among the most effective antibiotic drugs available and they are active mostly against gram positive bacteria. There are hardly any unfavourable side-effects on the patients. Active derivatives from penicillin can be obtained chemically and enzymatically.

Raw materials: Lactose, corn steep liquor, peanut meal.

Technology: Established and available; aerobic fermentation, fully aseptic.

Experience required: Considerable knowledge and experience.

Tetracyclines

Uses: Antibiotics (wide range).

Raw materials: Soya-bean meal, peanut meal, yeast extract, corn steep liquor, starch, sucrose.

Technology: Established and available; aerobic fermentation, fully aseptic.

Experience required: Considerable knowledge and experience.

(4) Nutritional supplements and flavouring agents

Riboflavin

The need to produce riboflavin, which is used as a food and feed supplement, will depend largely on the diet of the population and the composition of the staple foods.

Raw materials: Ground lentils, pancreatic digest of gelatin, molasses, sucrose; glycine is a critical ingredient.

Technology: Available but rather difficult; aero process gives highest yields; fully aseptic operation.

Lysine

General remarks as for riboflavin. Lysine is used for upgrading vegetable protein; competition from chemical synthesis is likely to develop.

Raw materials: Carbohydrate sources, biotin, corn steep liquor.

Technology: Not easily available; aerobic fermentation, fully aseptic.

Vitamin B_{12}

General remarks as for riboflavin.

Raw materials: Carbohydrate sources (molasses).

Technology: Established, comparatively easy; aerobic and anaerobic fermentation, fully aseptic.

5'-nucleotides

5'-nucleotides are used as a flavouring agent.

Raw materials: Carbohydrate sources.

Technology: Developing, not easily available; aerobic fermentation, fully aseptic.

Monosodium glutamate

Monosodium glutamate is used as a flavouring agent; competition from chemical synthesis is likely to develop.

Raw materials: Carbohydrate sources, biotin.

Technology: Developing, needs careful control, not easily available; aerobic fermentation, fully aseptic operation.

(5) Enzymes

Amylases

Amylases are essential for the fermentation process when starchy raw materials are used.

Raw materials: Bran of various grains, starch, potatoes.

By-products: None from bran, mycelium from liquid washes.

Technology: In developing countries amylases for saccharification of starchy materials during alcoholic fermentation are best produced on loose solid cultures of bran; the submerged process, more difficult, is usually used for production of amyloglucosidase.

Experience required: Careful control necessary in inoculum preparation.

Plant flexibility: Low, because of its small size.

There is scope for the production of several different products in a single plant, though not generally simultaneously. In reviewing the possibilities of setting up multi-purpose plants, a distinction has to be drawn between aerobic and anaerobic processes.

Aerobic processes

The following can be produced in multi-purpose plants, though some adaptation will usually be required when changing products:

Antibiotics	Citric acid
Vitamins	L-sorbose (for vitamin C)
Gluconic acid	Monosodium glutamate

Anaerobic processes

The following products can be produced in multi-purpose plants without adaptation provided that fully aseptic work is possible:

Ethanol	Lactic acid
Butanol	Vitamin B_{12} (by anaerobic bacteria)

Training

Needs

The experts that are sent to developing countries should make a careful assessment of the needs for training, particularly at the lower (technician, craftsman) levels. It is also essential that the technologists and engineers involved in setting up the plant and operating it should have practical experience in this field.

The group felt that the guaranteed availability of trained personnel over a long term is a condition sine qua non for establishing manufacture of fermentation products.

Methods

One of the primary methods of training is on-the-job training while the contractor is setting up the plant and supervising the project over a specified period of time. The contractor should be responsible for this training.

Considering that fermentation technology is a good means of introducing industrialization at many different levels, fermentation plants should provide extensive facilities for training technicians and craftsmen who would then move out to other industries. Trained personnel from certain existing plants, from sugar refineries, for example, can be employed by the first fermentation plants. In fact, a fermentation plant may serve two or more purposes, in that personnel can be trained for one type of fermentation product, while production of another takes place.

It is emphasized that engineers and technologists should have practical experience as well as theoretical knowledge and should be prepared to show the staff how to perform technical operations.

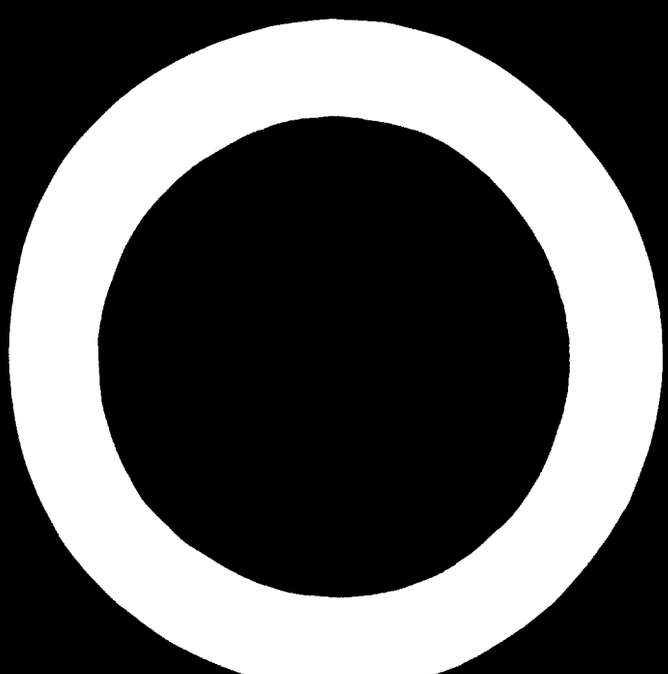
It is recommended that UNIDO seek co-operation with other United Nations organizations or agencies who are already operating such training schemes. UNIDO should also seek co-operation with other agencies in various countries (such as the British Council) that operate training schemes on various levels, and with research and development institutes in various countries.

Culture collections

A few regional culture collections should be established, which would supply cultures and transmit information and technical expertise relating to cultures. These should be located in the vicinity of fermentation plants or universities doing applied fermentation research. Staff members should be available capable of solving micro-biological problems in the plants of their regions. These centres might even supply inoculum to plants in the region. They would also be good places to train technicians in production control and techniques (determination of pH, product stability etc.). Training would last up to one year. Probably centres would be needed currently in Central Africa, India, the Middle East, North Africa and South America.

The Culture Collection Section of the International Association of Microbiological Societies invites the participation of

international organizations, such as UNIDO, in its programmes. It would also welcome suggestions from such organizations as to means by which it might aid science and technology in developing countries.



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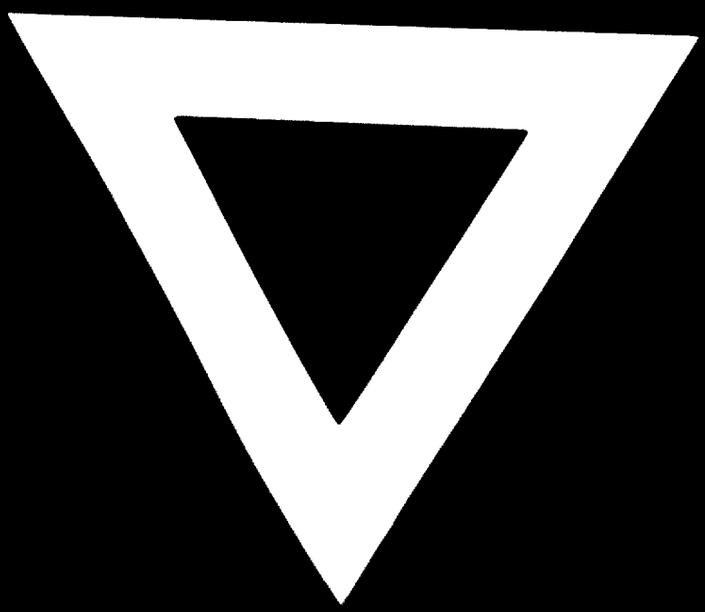

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