A SYSTEMS ANALYSIS APPROACH TO THE ASSESSMENT OF NON-CONVENTIONAL PROTEIN PRODUCTION TECHNOLOGIES

PROCEEDINGS OF A TASK FORCE MEETING
SOFIA, BULGARIA
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J. T. Worgan
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FOREWORD

The Food and Agriculture Program at IIASA focuses its research activities on understanding the nature and dimension of the world's food situation and problems, on exploring possible alternative policies which could improve the present situation in the short and long term, and on investigating the consequences of such policies at various levels - global, national and regional - and in various time horizons.

One part of the research activities focused on investigations of alternative paths of technology transformation in agriculture with respect to resource limitations and environmental consequences in the long term.

In this context an assessment of the possible impact on global and regional supplies of food of new, non-traditional technologies for the production of protein has been considered important and a series of Task Force Meetings were organized.

At previous Task Force Meetings held at IIASA and Tbilisi, U.S.S.R. the technologies for protein production were reviewed and a worldwide collaborative network established for the collection of data. The main theme of the Third Task Force Meeting held in Sofia, was on the qualitative and quantitative aspects which will influence the contribution that new technologies may make to food supplies within the period of the next twenty years.

Here Dr. Worgan has presented the papers submitted to the Third Task Force Meeting held in Sofia in October 1982, that was organized jointly by the Food and Agriculture Program of IIASA, the Bulgarian National Committee for Applied Systems Analysis and Management, and the University of Sofia.

Kirit S. Parikh
Program Leader
Food and Agriculture Program

ACKNOWLEDGEMENTS

The Editor is grateful to all those who contributed to the Task Force Meeting whether by formal presentation or through participation in the discussions.

Grateful acknowledgement is made to the Bulgarian National Committee for Applied Systems Analysis, to the University of Sofia and to Dr. J. Hirs, Professor G. Mikeladze and Professor S. Münch who as members of the Program Committee were responsible for the preliminary planning of the meeting.

Special thanks are due to Professor Ch. Panayotov and his staff of the University of Sofia for their hospitality to the participants and for the time and effort they spent in the preparation of such a well organized meeting.

These proceedings would not have been possible without the cooperation of Ms Cynthia Enzlberger who assisted with the smooth running of the meeting, coordinated all the subsequent correspondence and typed the final manuscript.

J. T. Worgan
Editor

OPENING ADDRESS

Dr. Yanko Markov
Minister of Forestry and Forest Industry, Bulgaria.

Dear Ladies and Gentlemen,

I am particularly pleased to greet you as participants in the Third IIASA meeting on a "Systems Analytical Approach to the Assessment of Non-Conventional Protein Production Technologies" which is being held in our country.

The Government of the People's Republic of Bulgaria highly appreciates the noble purposes of the scientists, gathered through the endeavours of the International Institute for Applied Systems Analysis, (IIASA) to revolutionize the technologies for obtaining protein from different sources of raw materials using biotechnological methods.

Forests cover one third of Bulgaria's national territory and play a significant role in the economy. This explains the interest of the Ministry of Forestry and Forest Industry in methods of efficiently converting waste wood materials and significant quantities of grass via biotransformation into high protein fodder.

Your Task Force Meeting once again confirms the great political and scientific role that furthering international collaboration, can play in guaranteeing and stabilizing peace in our world today.

I would like to wish you continued success in your noble endeavours for the prosperity and happiness of mankind.

OPENING ADDRESS

Prof. D. Davidov
President
Bulgarian National Committee for Applied Systems Analysis

Dear Participants,

I am honoured to greet you at the opening of the Third IIASA Task Force Meeting held to discuss ongoing research into non-conventional ways of solving the protein problems. After the first meeting in Vienna in 1980, and the second in Tbilisi in 1981, it is probable that now you will make further progress in finding new solutions to one of the basic programs of IIASA which is now entering its concluding phase. We hope that you will make valuable suggestions as to our further collaboration in this field.

Bulgaria is an active member of IIASA and has a particular interest in the successful conclusion of this program which you are participating in. Our National Committee of Applied Systems Analysis has several experimental projects for rational self-sufficient complexes based on non-waste technologies and is happy to shares its experiences in this trend with the other members. We would like to participate more actively in this program at IIASA and its realization over the next two to three years.

It is probable that other forms of collaboration besides these annual meetings will be necessary, such as consultations on particular problems, and participation of various specialists over longer periods of time to work on the general plans, analysis, conclusions, etc.

We hope that all countries concerned will take into consideration the difficulties we meet and will make the necessary efforts, including the financial ones, in order that our mutual collaboration may continue to enable us to finalize our results. The very positive outcomes of the Energy Program at IIASA, which has been highly appraised by many countries, provides us with reasons for optimism in this field too.

We are sure that you will make the necessary endeavours in your country to find the optimal solutions for the successful organization of this task and will help in the generalization of the results in the final stage of the mutual program.

I would like to wish you successful meeting and a pleasant time in our hospitable country.

OPENING ADDRESS

Prof. G. Bliznakov
Rector of Sofia University
Full Member of the Bulgarian Academy of Sciences

Dear Guests,

It is my pleasure to welcome you to Sofia University, which is honoured to host the Third IIASA Task Force Meeting devoted to such an important world problem as the search for new methods of solving the world's protein deficiencies, and I am particularly pleased with the presence at this meeting of so many prominent scientists working in this strategic field. We fully appreciate the efforts of the International Institute of Applied Systems Analysis in integrating the efforts in many countries in this direction, and we are making our utmost endeavours to help it.

The organization of this meeting in Bulgaria's largest university is a recognition of our modest efforts with respect to the creation of non-conventional tecnologies to obtain new protein sources. Our biologists and chemists have recently been paying more attention to this problem, and the exchange of experiences with others is of great importance. Biotechnology is a new interesting interdisciplinary field and we look forward to having successful results and only with mutual and complex efforts can we advance quickly. As a chemist, I appreciate the difficulties facing scientists in this exploratory work on new nonconventional technologies both in this and in other important fields. These technologies are of great economic and social significance and will resolve several of the most important problems of contemporary mankind.

Bulgaria is a country with a highly developed plant breeding, animal breeding, food and microbiological industry. In spite of the above mentioned, our future and optimum demands cannot be met by only traditional technologies of agricultural production

For this reason, two year ago we began a national program to resolve the protein problem involving many scientists from the University. We are convinced that our combined efforts will enable us to find the most optimal solutions inspite of many difficulties.

I would like to wish you rewarding discussions during this meeting and every success in your future activities for the benefit of mankind.

OPENING ADDRESS

Prof. Ing. Christo Panayotov
Prorector of Sofia University
and
Holder of the Chair for Engineering Biology and
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Dear Colleagues,

I am happy to meet you once again at our third consecutive IIASA meeting on Non-conventional Protein Production Technologies. We have just become accustomed to these occasions and now we have to conclude with their results. But scientists are inventive and will probably find other reasons to meet each other again concerning this and other problems.

Our present meeting is of great importance, and we have to generalize our current experience and to find other methods and means for our future work in this direction as well as discussing our present problems. We shall prepare these points during the discussions of the reports as well as at the concluding discussion when we shall accept recommendations for the next stages of the program.

Much to our regret, some prominent participants were unable to take part in our meeting, among them Prof. Parikh and Dr. Hirs, Program and Deputy Program Leaders, of IIASA's Food and Agriculture Program. However, Prof. Munch, the main executor of this activity will represent them.

On behalf of the Program Committee I inaugurate IIASA's Third Task Force Meeting on a Systems Analytical Approach to the Assessment of Non-Conventional Protein Production Technologies, and wish you a successful meeting and rewarding results.

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CONTENTS

SOME ASPECTS OF IDENTIFYING PROTEIN DEMAND ON A GLOBAL AND REGIONAL SCALE

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The problem of improving the world food situation and ensuring food supplies, especially for the fast-growing population in the developing countries, is among the most most urgent issues of the 1980s and 1990s. Sufficient food supplies for the under-privileged masses plagued and threatened by hunger and malnutrition is not only a techno-economic problem, but is above all, a political and social one.

The need for a diet adequate in terms of quantity and qualitative composition implies guiding the structure of food production and food supplies in accordance with these requirements. A diet can be regarded to be of full value when:

- it meets the energy requirements of the organism,
- it contains, in sufficient quantity as well as in the right proportions and in utilizable form, all nutrient elements needed for building up and maintaining the body substance and for the maintenance of all vital functions as well as for safeguarding resistance against physical and psychical stress.

Thus, adequate nutrition must be regarded not only from the point of view of quantity, but also of quality. Therefore crucial importance should be attached, both to the assessment of food requirements and to the knowledge of specific functions of individual nutrients, which provide essentials not only for the evaluation of the present food situation, but also prognostic considerations on the improvement of the global and regional food situation. This aspect is important because there is considerable variation in the required energy and nutrient intake, as influenced by age, sex, and physical activity.

Among the nutrients required, proteins play a particular role in providing a diet of full value, because:

- protein deficiency seriously hampers growth and physical development of children and adolescents as well as the health and vigour of all population groups, especially pregnant and nursing women,

  individual food items not only have different protein contents, but are characterized by a high degree of variation of protein quality, which has a bearing on the structure of food production and consumption patterns,
- protein is a major nutrient influenced by income. With rising income there is an increase in total protein intake and a gradual shift to more animal protein consumption (Figure 1),

- food habits or traditions frequently involve low protein food intake of certain population groups; this is particularly true for Developing Countries,
- to increase the output of food protein has proved more difficult compared to the production of food energy.

The particular status of protein in human nutrition as well as the question of assessing quantitative and qualitative requirements have been under the consideration of nutritional experts for a long time. Their assertions are highly relevant for practical purposes as they provide fundamentals suitable for appraising requirements as to the production and distribution of high-protein food on a scientific basis. This is true for both the so-called traditional foodstuffs and protein from non-conventional sources under discussion at this meeting.

Between 1949 and 1971, FAO and WHO set up 5 expert groups in order to review the standards determining energy and nutritional needs of the human organism. On the basis of more recent calculations, the 1971 expert group reduced the physiologically necessary protein needs and modified the "ideal" amino acid pattern of reference protein. As to the protein intake, the recommended amount of 0.71 grams of reference protein per kilogram of body weight of adult persons was lowered to 0.52 grams per kg of body weight in females and 0.57 grams in males. No essential changes were made in protein allowances for age groups up to 12 years and for pregnant and nursing women.

The recent standards set by FAO/WHO entail a sizable reduction of average protein needs based on "local" proteins. Whilst in the 1960s these requirements were estimated to be 61 grams (global average), the standards set by the FAO/WHO expert team in 1971 arrived at a figure of only 38 grams, i.e., a reduction of 38%. The re-evaluation of protein requirements gave rise to changed appraisal of the food situation in developing countries. Instead of a partially considerable protein deficit which had been noted for many years, pure statistics revealed a surplus averaging about 50% for the total of Developing Countries.

Different positions have been taken with regard to the re-evaluation of protein needs by the FAO/WHO expert team. Many nutritionalists point out that a precise experimental assessment can be made only by referring to a protein balance minimum, i.e., "the minimum amount of food protein by which a balance of nitrogen losses and protein supply in the form of raw protein can be barely attained" (Kofranyi, 1972).

There are also objections that FAO/WHO tests were carried out on well-fed people in industrialized countries while a direct measurement of minimum protein needs in Developing Countries is missing. The effects of stress and diseases which are known to increase protein requirements considerably, were not taken into account in experiments destined to assess protein needs. We can note an attitude towards recent standards which is, at least, sceptical, particularly when national standards are compared with FAO/WHO figures. In this connection, reference can be made to the protein intake recommendations set by the U.S. National Academy of Sciences and the National Research Council and by the Canadian Bureau of Nutritional Sciences, respectively.

As can be seen in Table 1, recommendations set in the U.S. and Canada for all age and sex groups are above FAO/WHO standards, irrespective of certain differences between both countries. In the FRG the "German Society for Nutrition Sciences" recommended an average daily per-capita intake of 58.5 grams of protein, based on an allowance of 0.9 grams plus 20% added per kg of body weight. The addition of 20% calculated on minimum intake is being regarded as

a compensation for extraordinary protein losses (Soeder and Kraut, 1975).

As a whole, one can assume that sufficient supplies of food energy for all population groups are a basic prerequisite for solving the food problem. For economic reasons, protein should not be substituted for high-energy foods because they would merely act as energy sources fulfilling the function of cheap carbohydrates to make up for the lack of energy in the diet. Considering this aspect it was a reasonable step to think over and to revise the appraisal of the food problem which, in the 1950s and 1960s was predominately regarded to be a problem of protein shortage. On the other hand, the tendency of "fading out" the protein issue from the food problem which became apparent in the early 1970s must be seriously questioned. Such a view cannot be justified when considering the availability of food protein in several countries with characteristic consumption patterns and to specific population groups--an aspect we must focus on particularly. Moreover it is to be doubted if recent FAO/WHO requirement standards are in line with actual conditions and needs. A striking fact is that requirement standards set for industrialized countries were higher than those for Developing Countries, though we must bear in mind that:

- the population structure of Developing Countries shows a greater proportion of children, adolescents, pregnant and nursing women, i.e., of groups needing diets of high protein content, than in Developed Countries,
- in industrialized countries local protein foods consist largely of animal products, i.e., of food items characterized, on an average, by a higher biological value than those of vegetable origin.

Taking into account that the variation of nutritional levels caused by social conditions in Developing Countries is, as a rule, much more marked than in industrial nations, we must rate the average protein standards recommended by FAO/WHO as being too low. At most, they may be regarded as minimum nutritional requirements, but not as recommended food intake or nutritional standards.

Calculations of future demand on a global and regional scale constitute a major problem, in view of the widely diverging views on minimum protein requirements. Taking FAO/WHO standards as a guideline, an increase in demand of 60% can be expected for Developing Countries in the 1980-2000 period, this increase being attributable only to the projected growth of population. This figure must be further increased to take into consideration the likely effects of overall economic developments and of the social changes associated with them (urbanization, economic growth, increase in purchasing power, etc.). These facts will entail significant changes of consumption patterns, a main feature of which is a growing share of high-value protein food in diets.

Bearing in mind these and other factors of uncertainty for any protein demand projection, we can make the following general assertions:

An increase in demand will take place mainly in the Developing Countries. Generally, a considerable rise of food demand in terms of quantity must be expected. Proceeding from the assumption that the majority of the Developing Countries will not be able to satisfy growing demands by larger imports and that such a trend is undesirable from the economic point of view, national strategic approaches should predominantly aim at boosting domestic food production and reducing losses during harvesting and storage. As to the improvement of the protein balance, the following steps can be taken in the area of conventional foods:

- putting new land into production,
- diversification of production patterns in food crops with special regard to an increased cultivation of high-protein species,
- breeding efforts for raising and stabilizing the yields of legumes, for increasing the protein content of crops and for improving protein in cereals and some other food crops,
- integration of crop cultivation and animal husbandry,
- increased use for human consumption of high-protein residues from vegetable oil production,
- greater use of fish, including fish protein concentrates, for human nutrition.

The increased demand will have to be satisfied, to a considerable degree, by vegetable products. Otherwise we might be faced with a further sizeable growth of the extent of hunger and malnutrition. At the same time, we may expect an overall increasing demand for animal products which will show, however, very marked regional differences. Unless the purchasing power of the population groups which have been, so far, in an under-privileged situation, is raised, a substantial aggravation of the disproportion in nutritional levels is likely to occur. These negative impacts might be intensified if cereals as well as other crops so far used for human consumption, are to be used for a significant increase in animal production.

In the majority of industrially developed countries, principal changes in the share of energy and protein consumption are not likely to occur within the 10-15 years to come. Certain changes in consumption patterns will probably occur, which could be attributed to the following factors:

- shifts within the energy-protein balance towards an increase in animal protein foods in certain countries where consumption of vegetable protein has, so far, been relatively high. Such a trend can be expected to take place, for example, in the USSR as the recently announced National Food Program is gradually implemented.
- stagnating or declining demand for expensive protein foods, particularly for highly-priced cuts of meat, assuming that in industrialized market economies there will persist high levels of unemployment, and real incomes of major parts of the population will go down,
- changes in the structure of the consumption of proteins of high biological value when, in a growing number of industrial countries, there is an increasing supply of relatively low-priced vegetable or, in some cases, even microprotein based foods, especially of meat analogues. Such changes could be stimulated by both an increasing demand for cheaper protein foods and promotional measures sponsored by nutritional policies (including propagating certain products in the mass media).

In the context of this basic tendency of future demand trends the issue of the potential impact of non-conventional proteins in global and regional nutrition strategies also needs to be investigated. There are two aspects to be taken into account:

- an increasing use of non-conventional proteins for feeding animals (mainly non-ruminants) could help to release for human nutrition, grains, soybeans and other foods of high value,
- non-conventional proteins could be employed for nourishing people directly, subject to their specific suitability for that purpose (absolute safety to health, digestibility, acceptance by consumers, etc.).

Though we have to start from the assumption that non-conventional proteins will be used within the next 10-15 years predominately for animal feeding, the second variant is surely worth considering, at least when prognostic reflections are being made because an excessive increase of animal production, at the expense of basic nutritional needs, does not seem to be a desirable target.

The scale on which non-conventional protein supplies will be adopted for feeding people and animals, respectively, and the pace at which this will occur, is conditioned above all by economic factors or rather by the competitiveness of those products, as compared to conventional animal and vegetable products (fish meal, soybean meal, skimmed milk, etc.). In addition, certain standards must be met with respect to quality. Considering this and making allowance for a more intensive utilization of local resources for improving the nutritional situation in developing countries, more attention should be paid, finally, to the development of relatively unsophisticated technological variants.

Table 1. Recommended Daily Protein Allowances

Class	Age in years	Weight (kg)	Protein (in grams) per day		
			FAO/WHO	USA	Canada
Infants	0-1	3-9	1.9*	2-2.2*	1.9-2.2*
Children	1-9	10-30	14-25	20-39	18-37
Adolescents:					
Male	10-19	31-65	16-38	40-54	38-54
Female	10-19	31-54	26-30	40-48	38-43
Adults:					
Male	20-80	65-70	37	56	56
Female	20-80	55-58	29	46	41
• Pregnant			38	76	61
• Nursing			46	66	65

* grams protein per kg body weight

Source: Ehrlich P.R. Ecoscience, San Francisco, 1977. p.302

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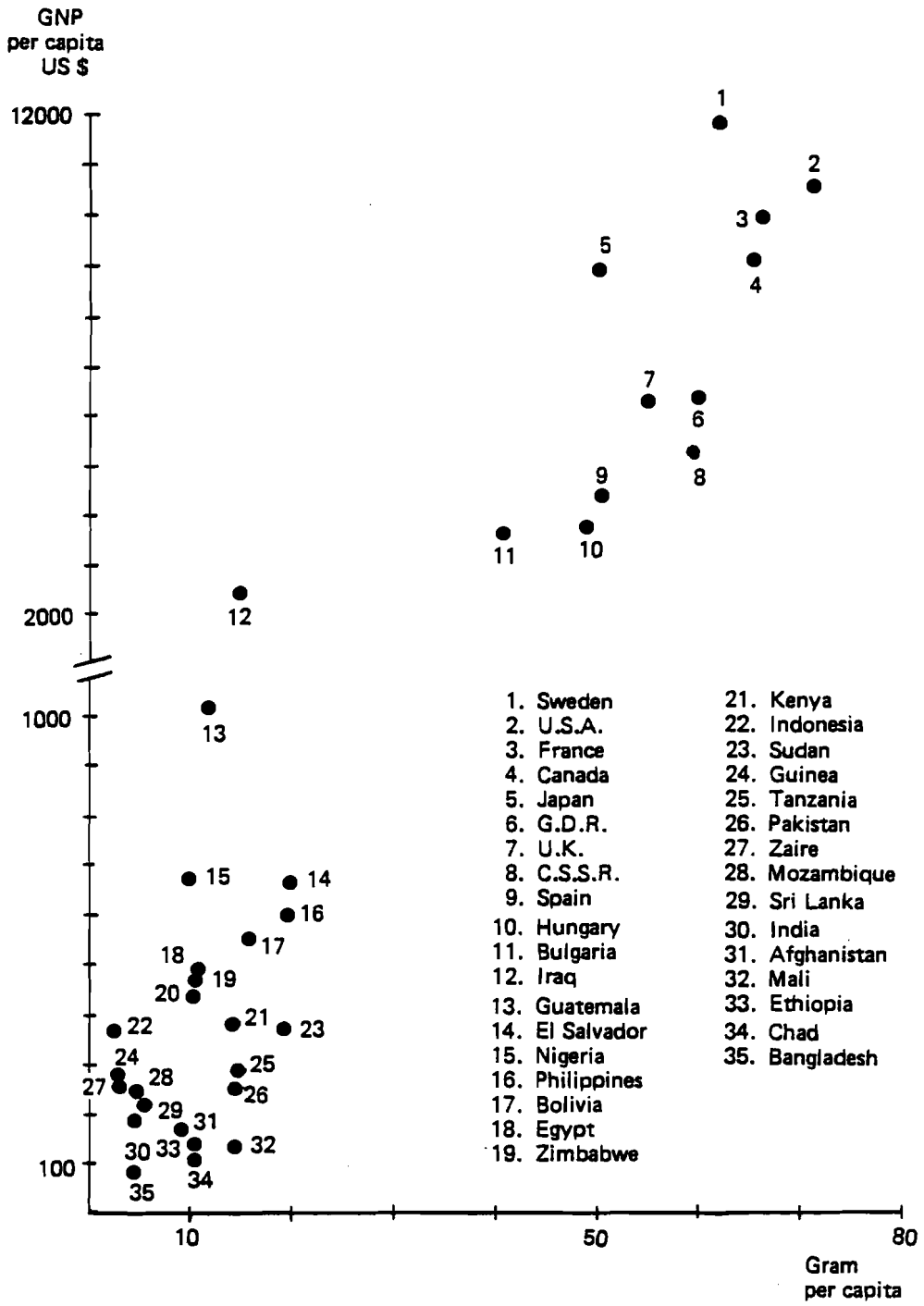

Fig. 1. Animal protein intake related to income levels (1978-80)

THE COMPARATIVE TECHNO-ECONOMIC STUDY OF CONVENTIONAL AND NONCONVENIONAL TECHNOLOGIES FOR FOOD PRODUCTION

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1. Biological Efficiency

Broadly speaking, we can define biological efficiency as efficiency of biological processes, even including such complicated combinations as ecosystems. There is no essential difference in applying the concept of efficiency to biology than to other fields. Similarly, the results of biological processes may also be non-biological, a biological efficiency ratio could use physical or chemical units. Generally, efficiency can most usefully be defined as the ratio of output to input:

$$E = \frac{O}{I}$$

where:

O represents a chosen output, and
I represents a chosen input.

Such a ratio can apply to innumberable combinations of output and input and each of these can be expressed in many different terms, some of them allowing several different outputs or inputs to be combined. The general concept is clear and obviously of value, and part of its value lies in its applicability to a great number of different or particular cases.

Once our interest has been specified, however, the most relevant ratio will be obvious. Particular versions are often named, such as energetic efficiency, relating energy output to energy input. It should be kept in mind that biological systems commonly use non biological inputs, such as solar radiation and that some of the most relevant efficiency ratios will be based on them. Agriculture for example, requires a source of energy costing much less than the energy value of its products. Of course, it also produces many other things as well as energy. Even so, the support energy to agriculture is very large today and a very large yield is imperative. This requires special calculating in each particular case.

It is clear that by using modern technologies, productivity per hour of labour has risen nearly a hundredfold, yields nearly threefold, but energy requirements have increased more than tenfold and the efficiency ratio has dropped to about threefold. The interpretation of this result is difficult to evaluate in any tems, because there is not a universal basis of comparison. The view

Table 1. Efficiency ratio in primitive and modern maize cultivation

	Primitive Mexico		Modern USA	
Inputs	amount/ha	MJ/ha	amount ha	MJ/ha
labor	1144hrs	2227	12hrs	23
seeds	10.4kg	1140	21kg	2198
machines			31kg	2336
oil			112 l	5352
N fertilizer			28kg	7878
P fertilizer			72kg	904
K fertilizer			80kg	536
limestone			100kg	132
irrigation			780t	3226
insecticides			1kg	364
herbicides			2kg	836
drying				1785
electricity				1591
transportation			136kg	146
Total input		**3367**		**27350**
Output (Maize)	**1994kg**	**28890**	**5360kg**	**80200**
Efficiency ratio		**8.60**		**2,93**

differs in value to different people and countries and at different times. What you are going to select depends on many other factors. Some products are generally more valuable than others, although prices, both relative and absolute, may fluctuate quite widely.

An example of the effect the nature of the product may have on its price is given in Table 2 where the difference between the price of 1kg of protein from plant and animal sources is compared.

Table 2. Relative prices of pure protein (100%) by different sources

Product	Price/ton (US dollars)	% Protein	kg Protein/ton	Price/kg Protein (US dollars)
Wheat	150	12	120	1.23
Maize	130	10	100	1.30
Soyabean	340	42	420	0.81
Beef	1300	16	160	8.12
Lamb	1700	14	140	12.14
Pork	1100	11	110	10.00
SCP	750	50	500	1.50

So it is quite possible for a farmer to produce less food for people, because his purpose is then monetary gain or meat production, although it is well known that more people can be fed per unit of land if it is used for crop production.

The economic adjustment of such situations depend upon the need for food being adequately expressed as a demand, but this cannot occur when hungry people are also poor.

2. Efficiency in Crop Production

The grain crops are of the greatest importance in feeding the world's population. It has been calculated that the yield of world's cereal production, represents enough protein to supply 100g per head per day to a population of three billion people.

The most important resources for cereal production, are land and power--the key inputs for efficiency calculations. The amount produced per unit of land, per unit of time, per unit of labour, per unit of support energy, have their specific values.

Table 3. The output of energy and protein per unit of labour per hour

Product	Yield kg	Protein kg	Energy MJ
Wheat	300	37.2	5520
Maize	417	40.9	7923
Barley	252	27.2	4612
Sorghum	386	41.7	7257
Alfalfa (dry)	526	86.7	4986

In this case the best source of protein is alfalfa and for energy maize.

Table 4. The output of energy and protein per unit (GJ) of support energy

Product	Energy (GJ)	Protein (kg)
Wheat	4.2	28
Maize	2.9	14
Barley	3.6	21
Sorghum	5.8	33
Alfalfa (dry)	6.2	107

In this case the best efficiency ratio without doubt is alfalfa, which is very important economically.

The major measures of efficiency are thus output of energy and protein per unit of land, labour and support energy. This clash of efficiencies means that there are optimum values for size and structure of enterprises. They can be valued and it is quite clear that preferences, beliefs and traditions could change in the future. The main factors affecting yield therefore vary with the environment.

Table 5. Output of energy and protein per unit of land/ha

Product	Yield kg	Protein kg	Energy 10^6 KJ
Wheat	4120	494	57
Maize	5400	485	80
Rice	6160	462	94
Sorghum	3030	344	44
Soybean	1880	640	32
Potato	34380	722	83
Spinach	11200	358	12
Tomato	49620	496	42
Apple	17920	36	40
Orange	19040	193	28
Bean	1460	325	21
Peanut	3720	320	64
Alfafa (dry)	6830	1127	65

In this case alfalfa is also the best source of protein and one of the best for energy together with maize, rice, potato, wheat and peanut.

3. Efficiency in Meat Production

The animals vary enormously in size and productivity. The major resources used in animal production are land, feed, labour, and capital. Although the efficiency with which the major resources are used is important, no single efficiency ratio can be regarded as of over-riding importance, and judgement still has to be based on an examination of a whole range of them.

Table 6. Efficiency value in relation to feed dry matter

Animal	Feed (kg/dry matter)	Carcass (kg)	Efficency$^+$
Cattle	3005	257	8.6
Sheep	108-136	19-18	17.6-13.2
Pigs			
pork	93.6	44.8	47.9
bacon	174.8	67.3	38.5
Rabbits	2.37	0.99	42.0
Hens			
broilers	4.00	1.45	36.0

$$E^+ = \frac{\text{Carcass output (kg)}}{\text{Feed input (kg dry matter)}} \times 100$$

In any event, the efficiency with which animals use their feed does not necessarily indicate efficiency of land use, since this also depends upon the amount of feed grown per unit of land and this varies with the nature of the land. Thus a feed with a high feed conversion efficiency for pigs tells us nothing about the efficiency with which pigs could use land that cannot grow that particular

feed. Furthermore, although land is often regarded as the ultimate limited resource, "land" is not a homogeneous commodity and some land is nearer to where the food is required, some land needs more inputs than other land, and all the land could grow anything if the inputs were high enough and included sufficient control over the environment. Usually, as shown in the example given above, the most effective are pigs.

Table 7. The efficiency of land use for meat production

Animal	$E = \frac{\text{kg carcass}}{\text{ha land}}$
Cattle	598
Sheep	429
Broiler hens	852
Rabbits	932
Pigs(pork)	812

In this case the highest efficiency ratios are for rabbits, followed by hens.

Table 8. The efficiency on energy basis

Animal	$E = \frac{\text{energy output}}{\text{energy input}}$
Cows	0.11
Sheep	0.39
Rabbits	0.03
Hens	0.04

In this case sheep have the highest energy ratio.

Meat production is thus characterized by this increasing maintenance burden and generally, this is not accompanied by proportionate increases in rate of growth. The result is that individual meat-producing animals tend to become less efficient in the use of feed as they grow larger. Clearly, there is no possibility then of slaughtering them before such efficiency declines. However, the efficiency of the whole family unit or population does not decline in this way.

4. Efficiency in Microbial Production (SCP)

Micro-organisms have been considered to have two major aavantages: very high rates of production per unit of time and very high protein content. It is well known that 500kg bull takes a day to lay down 0.5kg of protein, while 500kg of yeast produces 50 tons in the same period of time - an advantage of 100,000 to one. The speed at which production can proceed in respect to efficiency is most clearly demonstrated with single-cell protein.

One measure of rate of production is "protein doubling time" (the time required to double initial weight).

Table 9. Rate of conversion

Micro-organisms	Doubling Time (hours)
Yeasts	3-5
Fungi	2
Bacteria	0.8-4.7

The protein content of micro-organisms appear very high.

Table 10. The protein content by SCP producers

Micro-organisms	Protein %
Fungi	20-45
Yeasts	40-60
Algae	30-60
Bacteria	50-75

But some reservations have to be made about the biological value of this protein. Some of the protein may be insoluble cell wall fractions that are only digested by humans to a very limited extent and some, such as yeasts, contain very high levels of nucleic acids and need extraction.

Table 11. Biological efficiency of microbial protein

Micro-organisms	BEP index
Yeasts	5.3
Fungi	3.1
Bacteria	2.8

The greater the value of this BEP index, the lower is the efficiency of SCP.

In comparison with higher plants, algae for example have very favourable rates of dry matter production per hectare.

Table 12. Growth rates by algae and higher plants per year

Organism	Protein %	Dry Matter ha^{-1} / kg
Chlorella	50	$60-90 \cdot 10^3$
Soybeans	33	$1.5 \cdot 10^3$

Another advantage of SCP production is that it is based on quite different resources from those used for higher plant production. Lignocellulose wastes occur in vast quantities in many countries and can be converted by micro-organism into animal feed. It has been calculated that on a world basis the quantities of cellulosic waste (100,000 million tons per annum), the energy required to provide 100% of the 5.98×10^{10} kg, the world's annual protein need could be met by feeding only 5% of the world' s waste cellulose to animals.

Table 13. Production of crude protein per hectare

Production	Yield (dry matter kg/ha)	Crude Protein %	Crude Protein (kg/ha)
Plant			
Grass	12000	17.5	2100
Wheat	3780	12.4	470
Maize	3995	9.8	392
Barley	3240	10.8	350
Animal			
Cattle	360	14.8	53
Sheep	462	14.0	65
Pigs	875	11.9	105
Broilers	980	13.8	135
Rabbit	1511	19.3	292
Microbial			
Spirulina	48608	50.0	24304
Chlorella	31360	50.0	15680

In comparison to plant protein production per unit of land microbial protein is 40 - 60 times more productive and 200 to 300 times more productive than the animal protein.

5. The Relative Efficiency of Different Methods of Food Production

The quantitative relative efficiency of different means of food production can always be related to similar resources, because animal production must ultimately be based on plant or microbial production. However other qualitative aspects have also to be taken into account when considering food products. If potatoes are the required product, the efficiency of tomato production by comparison is irrelevant. This aspect is particularly important in relation to the production of animal products which nearly always appear much less efficient than crop production when considered from the point of view of quantitative output. Consumer acceptance also plays a part and can be measured in monetary terms.

Table 14. Production of protein expressed in monetary terms

Production	Output (tons/ha)	Price (US dollars/ton)	Value (US dollars/ha)
Plant			
Grass	12.0	82	984
Wheat	4.4	150	660
Maize	4.7	130	611
Barley	3.8	140	532
Animal			
Beef	0.44	1300	572
Lamb	0.46	1700	782
Pork	0.88	1100	968
Chicken	0.98	1000	980
Rabbit	1.50	1800	2700
Microbial			
Spirulina	48	700	33600
Chlorella	32	600	19200

In this case monetary efficiency is almost the same for animal and plant production, but compared to microbial production by comparison is 20 - 50 times more effective than the others. The possibilities in the world are different, traditions are still strong, but it is quite possible for microbial protein to become a major source of feed and eventually food. With the latest achievements in genetic technology it is certain that progress in microbial productivity in the near future will be doubled or even tripled.

For the hungry man the most important factor is the one which shows how many people could be supported by the production from one hectare of land for the most reasonable price:

Table 15. Number of people whose annual needs could be met

Production	Protein (kg/ha)	Energy (MJ/ha)	No. of people Protein	No. of people Energy
Plant				
Cabbage	816	105000	34	23
Potato	522	102080	22	22
Wheat	470	69534	20	15
Maize	392	75905	16	17
Rice	375	87768	16	19
Barley	350	59274	15	13
Animal				
Beef	65	4796	3	1
Lamb	65	7486	3	2
Pork	105	14438	4	3
Rabbit	292	13251	12	3
Chicken	135	7056	6	2
Microbial				
Spirulina	24304	1032100	927	458
Chlorella	15680	786300	642	396

In this case the SCP is 20 to 30 times more effective than plant protein, and 200 - 300 times more effective than animal protein. The underlying assumptions are:

(a) that more people could be fed on grain

(b) that animal products are not strictly necessary, and

(c) that SCP has a bright future.

FUTURE DEVELOPMENT OF NON-CONVENTIONAL PROTEIN TECHNOLOGIES AND PROBLEMS OF IMPLEMENTATION

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1. Introduction

The aim of the Food and Agriculture Program of IIASA is to assess the food supply situation within the period of the next 20 years. At previous Task Force meetings it was concluded that maintaining adequate protein supplies to meet the demand of the increasing population would be the most critical aspect and proceedings at these meetings were therefore concerned with methods by which supplies might be increased. One aspect of the FAP program is involved with agriculture, however at this, and previous Task Force meetings, alternative methods of protein production are considered. The term Non-conventional Protein Production Technologies has been used to describe these methods and can be defined as methods for Protein pro- duction for human or livestock consumption which do not, at the present time, make any significant contribution to supplies.

The world population in 1980 was approximately 4,400 million (FAO, 1979a). Protein intake per capita per day for the period 1975-77 is estimated to have been 69.2g consisting of 44.8g from plant sources and 24.4g from animal sources (FAO, 1979b). The same pattern of consumption can be assumed for 1980. Thus even without making any allowance for storage losses, wastage and the additional quantities of primary sources of protein required to produce animal protein, global production of protein in 1980 was certainly more than one hundred and eleven million tons. Any new technology will, therefore, need to be capable of providing at least one million tons per year if it is to make any significant contribution to global supplies. Some methods, which may not be capable of producing this quantity, may still be appropriate if they help to provide protein self sufficiency on a regional scale.

2. Nutritional Aspects

A new technology for protein production can only be justified if the product is of good nutritional quality. Before deciding to proceed beyond initial laboratory investigations an assessment should be made of the nutritional quality of the protein. This assessment should include an analysis of the essential amino acid composition, and determinations of the digestibility and biological value. A good indication of both the digestibility and biological value can be obtained by feeding trials to measure the net protein utilization value (NPU) or the protein efficiency ratio (PER).

3. Safety Aspects

The most critical limitation on whether a new technology can be put into practice is the requirement to establish the safety of the product. New protein sources for human consumption require a lengthy and extensive testing programme over a period of 4 to 5 years. Any trace of adverse effects will prevent the technology from being applied. Even in countries where government regulations may not prevent the distribution of the products, consumer resistance is a significant deterrent, without authoritative proof that the product is safe to eat. For livestock it is equally important that the protein source should not contain any harmful components. However, the testing conditions can be less stringent, not only because consumer resistance is of less importance, but because the lifespan of livestock is much shorter than that of human beings and the elaborate testing programme for components which only cause problems when consumed regularly over a period of years, are not so essential. Guidelines for the testing of new sources of protein for human (PAG, 1970)and livestock (PAG, 1976) consumption have been published by the United Nations.

Based on this limitation, non-conventional protein technologies can be divided into two categories:

A. Technologies in which the product is an established source of protein where the non-conventional aspect is involved in the method of production.

B. Technologies where the product is a protein source which has not previously been extensively consumed as either human food or livestock feed.

4. Technologies for the production of established sources of protein

The separation of proteins from agricultural and food industry wastes are in this category and several examples have been given at previous Task Force meetings (Hirs, ed. 1981; Hirs and Muench, eds. 1982). Leaf protein from some leafy crops when used for livestock feed may also be in this category, although for human consumption more stringent testing may be necessary. Several of the oilseed protein residues could also be included and since these technologies have not been dealt with at previous meetings, a brief summary is given below.

It has been estimated that 22 million tons of discarded or underutilised protein occurs each year in the oilseed residues (Altschul, 1970) Soybean meal is the main example of a product which has become extensively used for the feeding of livestock and for this application it can no longer be regarded as an unconventional protein source. The soyabean was originally grown in the USA for the extraction of oil and this is still the main product. The residue, after oil extraction, contains proteinase inhibitors, which have an anti-nutritional effect, and haemoglutenins which produce toxic symptoms. Both of these components are sensitive to heat and can be inactivated by heat processing conditions which do not cause any extensive damage to the nutritional value of the protein.

The recently developed technology for the texturing of soya protein into products which are similar to meat (Tombs, 1978) is still in the category of an unconventional technology, although production has expanded rapidly during the past few years. Although the technology does not directly increase protein supplies, it does produce a similar product to meat from less than one third of the primary protein source required for meat production and therefore has a corresponding beneficial effect on protein supplies. Other oilseed residues are also valuable sources of protein which could be extracted and used for human consumption. In the case of coconut for example, the established method for producing coconut oil is to dry the flesh of the coconut in the sun to give the product called copra. Because of the low standard of hygiene in the drying and

extraction processes, the residue, after oil extraction from the copra, is not fit for human consumption. It also has a high fibre content due to the removal of the oil and even the clean product is not suitable as a source of protein for child feeding. The technology has been developed to separate in one process from fresh coconut the oil, the fibre, and protein suitable for human consumption (Dendy and Timmins, 1973). Methods developed for the recovery of protein from most of the other oilseeds are summarized in the literature (Adair and Orr, 1967; UNIDO, 1974) and the application of these new technologies could make a significant contribution to protein supplies.

The production of amino acids by chemical (McPherson, 1972) or microbiological methods (Kinoshita, 1963) yields products which can be purified and are not, therefore, subject to the need for safety tests. In terms of quantitative supplies the amino acids are unlikely to make a significant contribution. However, the supplementation of foods or feeds deficient in the essential amino acids lysine and methionine can considerably increase their nutritional value and effectively reduce the quantity of protein required to meet physiological need. Technologies for the production of lysine and methionine are in use and an increase in supplies in the future could contribute to meeting the nutritional demand for protein in the future.

5. Technologies which yield proteins which are not established sources of protein as food or feed.

Microbial sources of protein produced by bacteria, fungi, yeasts or algae are the main category of the type of technology and several papers on this topic have been presented at previous Task Force meetings (Hirs, ed. 1981; Hirs and Muench, eds. 1982). The biomass of micro-organisms occurs in all natural environments and it is probable that most samples of food contain remnants of microbial cells. A higher proportion of microbial cell mass is present in foods prepared by microbiologicl processes. Beer, wine, bread, cheese and yoghurt are some examples and in some of the oriental foods such as tempeh and miso a considerable proportion of the product consists of microbial cell mass. The idea that man should eat micro-organisms is therefore not new, although it is only in relatively recent times that the technology has become available to produce the biomass of micro-organisms as a specific product. Because the products are novel possible sources of food or feed they need to undergo the extensive testing programme referred to above.

One exception to this aspect is the cell mass of the algal species Spirulina maxima, which has been found to accumulate in natural circumstances in alkali lakes in West Africa and in former times in Mexico. The cell mass, which contains 62% protein, has been harvested and used as a food for centuries without ill effects (Clement, Giddey and Menzi, 1967). The technology for the cultivation of Spirulina has been developed and the product is being marketed as a food product in the USA. Food yeast *(Candida utilis)* is a micro-organism for which the technology of production has been established for more than 50 years and is now accepted as a product which is safe to use for human consumption. The unconventional aspects of this technology relate to extending the range of raw materials on which the yeast can be grown and this should increase the supplies which could be made available in the future.

Two technologies have been developed to the stage where the products have undergone adequate testing programmes to allow them to be used as livestock feed. Production units are in operation and the products are being marketed. The Pekilo process involves the fungal species *Paecilomyces variottii*, the mycelium of which is grown on a liquid waste arising from the manufacture of

cellulose pulp and is being produced as an animal feed in Finland (Romantschuk, 1976). In the UK the ICI company are producing and marketing the cell mass of the bacterial species *Methylophilus methylotrophus* as the livestock feed called Pruteen. The bacteria is grown on methanol, manufactured from natural gas. The extent of the tests made on this product is indicated by the statement that data has been obtained from the feeding of 500,000 animals (Smith, 1980).

At the Task Force Meeting held in Tbilisi in August 1981, (Hirs and Muench, eds. 1982) it was announced that the mycelium of the fungal species *Fusarium graminearum* had been approved as a food product in the UK. A process for the production of the mycelium grown on starch has been developed, although to date no full scale production unit has been established. The yeast *Candida lipolytica* grown on petroleum in the process developed by British Petroleum, has undergone an extensive testing programme and its safety as an animal feed has been established. A plant to produce 100,000 tons a year has been constructed in Italy, but due to a decree issued by the Italian Government, this plant has been prevented from going into production (Gosling, 1977).

6. Energy Inputs

It is now generally accepted that the fossil fuels are a limited resource. However, even in the most energy intensive systems of food production in the UK and the USA less than 5% of total energy consumption is used to produce the food supply for the population (Worgan, 1975). Provided that new technologies do not require energy inputs which are considerably in excess of those used in agricultural production, energy supplies may not be such a serious deterrent to their adoption in the future as has been suggested.

7. Land Use

It is estimated that well over helf the arable land available is currently in use to supply the world population with food. In many areas arable land is being lost due to erosion and urban development. Land may therefore become a more critical resource than energy to maintain the food supply for the increasing population. Most of the Non-conventional Protein Technologies do not require additional arable land and this may be one of their main advantages in contributing to supplies in the future.

Although algal culture does require a greater area of land than other microbial methods, the process does not require arable land and need not therefore compete with agriculture for this resource. Leaf protein production is in competition with agriculture. However, with an appropriate cropping system in areas with a suitable climate, protein yields per unit area can be much greater than those of agricultural systems.

The production of substrates, grown specifically for microbial processes, will use land which could be in use for food production. This technology will therefore only be worthwhile if there are considerable advantages in the yield and quality of the protein produced.

8. Raw Materials

It has been established at previous Task Force meetings that vast quantities of wastes which occur annually are produced from agriculture, forestry, industrial processes and the processing of foods (Hirs, ed. 1981; Hirs and Muench, eds. 1982). Although account has to be taken of the logistics of collecting these wastes and maintaining an even flow to processing units, there are still large quantities which it would be possible to use. The application of technologies which use these wastes is not therefore likely to be limited by raw material

supplies.

Some of the processes which use fossil fuel raw materials may also continue to be feasible because of the high efficiency with which they operate. For example, more than 100 tons of yeast is obtained from 100 tons of petroleum raw material (Gosling, 1977) and in terms of efficient utilisation this may be a better return than using the petroleum for non essestial purposes. Methanol, used as the raw material in the ICI process for producing Pruteen, can be manufactured from the waste flare gases which occur in many of the world's oilfields.

9. Costs

Although the final price for food products are frequently influenced by artificial effects such as price controls and subsidies, the real cost is an approximate measure of the resources required for production. Technologies on which the estimated cost of production exceed the real cost of corresponding protein products produced from agriculture are unlikely to be put into practice at the present time. However, if protein shortages develop and agricultural costs become relatively more expensive, technologies in which the costs do not considerably exceed the cost of products from agricultural methods, could become viable production units in the future.

The number of stages in a technology does have a considerable influence on overall cost. For example a technology operating in 3 stages, each giving an 80% yield of the theoretical value, which is not unusual in practice, has an overall yield of only 51% and this will be reflected in the cost of unit output. In the paragraph above it has been suggested that fossil fuel supplies may not be a serious limitation, however, if the price of fossil fuels tends to continue to increase this will influence the relative cost of technologies with a high fossil fuel input.

Technologies in which the raw materials are wastes which cause pollution problems will have a cost advantage because the waste treatment cost can be offset against the cost of protein production. The Pekilo process for the production of fungal protein from cellulose pulp manufacturing waste is an example.

10. Time Scale

For technologies which are still at the stage of laboratory studies another 2 or 3 years will be needed to complete the investigations and correlate the information needed to proceed to pilot plant studies. The design, construction and solving the inevitable problems which arise, will involve a further 2 or 3 years to complete the investigations on a pilot plant scale. For products which are being considered for human consumption, to produce the quantities of material required and carry out the extensive testing programme, at least 5 years will be needed. For a livestock feed this period might be reduced to 3 years.

Even if everything upto this stage is proved to be satisfactory, obtaining the necessary finance or authoritive approval for proceeding to the stage of a production unit, may cause further delays. For the design and construction of a full scale production unit and to solve the problems of initiating the process, a 3 year period would probably be required. Finally, even if a high priority was given to the production programme, it would probably take another 3 years to reach the stage of producing 1 million tons of protein per year.

Thus, for a potential technology which is still in the laboratory stage of investigation, a period of at least 15 years will be required before the technology will be able to make a significant contribution to global supplies.

11. Acceptability of the Product

Even when a product has been confirmed to be safe to eat and the technology has been developed for its production, it must be converted into a form which people are prepared to accept as part of their regular diet. Food yeast, for example, although a good nutritional source of protein, is produced as an amorphous mass which is only suitable for inclusion in the diet when it is added in small proportions to other food products.

The technique of texturising which has been applied to soya protein, has been shown to be possible for other protein sources, including proteins recovered from wastes and to yeast and bacterial proteins. Although these possibilities have been demonstrated in the laboratory, they have not yet been developed on a production scale. Fungal mycelium has a characteristic texture which enables it to be converted directly into acceptable food products.

Investigations on this aspect should be made in parallel with pilot plant studies.

12. Personnel

The development and finally the operation of the technology on a production scale will require a wide range of skilled personnel. Trained staff will be needed to design, construct and operate the pilot plant and to carry out the extensive toxicity testing programme. Further skilled people will be required for establishing and operating the first production unit. For the final stage of extending the technology to several production units a proportionately large number of skilled personnel will be needed. A planned training programme should therefore be initiated during the earlier stages of development, to ensure that a lack of skilled personnel will not be a limiting factor when the expansion of the production programme is ready to proceed.

The aspects discussed above illustrate the wide variety of factors which need to be taken into account when developing a new technology to the stage where it can make a significant contribution to global protein supplies. The time scale is particularly important and if protein supplies do become critical it may be too late to initiate a programme of development. It is, therefore, suggested that new technologies should be developed to the stage of a first production unit. Priority can then be given to expanding the scale of production in the knowledge that there will be no difficulties with either the process or the product.

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ACTUAL SITUATION AND FUTURE TRENDS OF FOOD AND FEED PROTEIN PRODUCTION FROM AGRICULTURAL AND FOOD INDUSTRIAL WASTES AND BY-PRODUCTS

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1. Introduction

One of the most important problems of mankind today is to investigate new sources of food and feed, especially those containing protein.

The deficiency of protein-containing products is sharply felt all over the world. The "population explosion" has made traditional methods of protein production less effective in meeting demand and as a result of this protein deficiency is increasing.

The existing situation has turned scientists' attention to the wastes, by-products and to the world's supply of organic compounds as sources for obtaining protein substances, using non-traditional methods.

Agricultural and industrial wastes and by-products could play a leading part in the problem of decreasing protein deficiency. These wastes and by-products are a source that can be annually restored and exist in large quantities.

Protein from non-traditional sources can be used as a feed for animals or as food in the human diet. Feeding of livestock with protein containing products is less effective than their direct use as a protein addition. Besides, it can be applied for obtaining food analogues.

Thus, in order to use protein-containing wastes, first of all we have to solve two main questions: (1) protein extraction for food and (2) their usage as a protein-containing feed.

One of the most promising ways to do away with protein deficiency is by microbial protein production, because microorganisms, in productivity, surpass animals and plants several thousandfold.

Nowadays, yeast protein production obtained from wastes of oil-processing industry has been widely applied in the world. But the oil and gas resources in nature are limited.

In order to solve the problem of protein deficiency by means of proteins from non-traditional sources it is necessary to find the optimal solution for those factors which determine the successful solution of the given problem and to elaborate national, regional and global models, based on the status and interdependence of these factors. The above mentioned factors are: selection of raw materials, and the technology of obtaining and applying the protein products.

This presentation deals with our attempt to consider the status and prospects of the above mentioned questions in relation to these factors.

2. Raw Materials for Protein Food-stuff Production

To have a large capacity protein produciton it is obligatory to have the existing raw materials which can satisfy a whole number of factors. The main factor is - the quantity of raw material and the stability of its resources in relation to its quantity and quality. The second factor is - chemical and physico-mechanical properties of raw materials which define their safety, storability, transportability and processing technology.

The third one is - economical and social aspects of available raw material usage in relation to the above mentioned factors; the most promising are wastes and by-products of agricultural raw material processing (Fig. I).

The supply of raw material resources is related to agricultural production, characteristic for the given climatic and soil conditions as well as their traditional application.

At the same time we have to take into account uncultivated (wild) raw materials too, as an additional resource.

If a given agricultural crop can successfully be substituted then we should decide which of these should be considered while working out a model. It is equally applied to the usage of the raw materials. The technology of its application which presumably would not change in the given region at least over 10 - 15 years is the guideline. The technology of usage of some of the same raw materials may be different in various countries and depends upon national peculiarities, the technological level and some other factors. And that is why while drawing-up a national model differential approach is essential.

The chemical composition of wastes is the other important factor. The concept - chemical composition of wastes means the presence of constituents which are useful and digestible for microorganisms as well as the presence of toxic and anti-nutritional components, there is some correlation between these properties and the digestible and indigestible substances by animals. These data define safety, effectiveness of their usage as feed and the possibility for them to be used for microbial protein synthesis. From industrial production we have quite different wastes according to the type of technology.

According to physico-mechanical properties the wastes are divided into solid, fibrous, solid non-fibrous and liquid; consequently each needs a suitable technology and recycling.

It is necessary to classify vegetable agricultural food raw materials into main groups characterizing their biological peculiarity; and each of them must be divided into sub-groups, according to their usage.

In Figure 2 all kinds of vegetable agricultural food raw materials are divided into 5 groups: fruits, vegetables, cereals, oil-producing crops and leguminous crops.

To establish the kinds of wastes and by-products according to groups it is necessary to determine their usage in the fresh form and after industrial treatment. Depending on technological treatment, different raw materials are left for each group: when we deal with fruits it is obligatory to discuss, separately, their usage in the fresh form and their industrial treatment. From the application of fruits in the fresh form we have two kinds of wastes: wastes of fruit commodity treatment and trade wastes.

From industrial processing, there are entirely different types of waste. Of

course, this division has a general character as each category of raw material, given in Figure 2, consists of a large number of different kinds of raw material and technological aspects which defines their distinctive characteristic according to their chemical and physico-mechanical properties.

Depending on protein content, wastes and by-products are divided into two groups (Fig. 3). Wastes having high protein content are recommended for protein extraction for feed and food but protein microbiological synthesis can be carried out with these wastes. When we have a low protein content it is recommended to use raw material for microbiological synthesis, without preliminary extraction of protein substances.

3. Obtaining Protein Products by means of Non-traditional Technologies

All the non-traditional methods of obtaining protein substrates from vegetable raw materials can be divided into the following categories (Fig. 4):

1. Protein extraction from raw materials.
2. Protein biosynthesis by yeasts and obtaining from them protein isolates and amino acids.
3. Enrichment of wastes (used as a feed) by means of microbial protein.
4. Protein biosynthesis by using higher fungi.
5. Protein biosynthesis by means of microscopic fungi.
6. Protein biosynthesis by using bacteria.
7. Protein biosynthesis by means of symbiotic-methods using various micro-organisms.
8. SCP production from algae.

The extraction of protein substances from vegetable raw materials is used extensively. At presently, this method is the main non-traditional method of obtaining food protein. Mainly these proteins are applied in feed. Wastes of vegetable food protein production are used in cattle-breeding or are not used at all. Such a technological approach is no longer acceptable. It is more advisable to use solid and liquid wastes as a substrate for microbial protein production.

Yeasts are the most productive micro-organisms for the protein biosynthesis. The grown biomass is used as a feed. Many authors propose protein isolates, extracted from them, in feed.

Amino-acids are extracted from yeast autolysates and are offered for food. The positive character of yeast production is their short cultivation cycle with 6 - 10 hours duration, high productivity of protein, 50-60%, and low prices. It does not require expensive equipment nor completely sterile conditions. The negative character of this process is a high content of RNA, that causes their limited application as well as the necessity of preliminary polysaccharide hydrolysis. Polysaccharides occur in large amounts in agricultural wastes.

Higher fungi are excellent resources of non-traditional food protein, but their cultivation is possible only on a limited number of substrates. Their application is limited by high costs and specificities in relation to organoleptic and rheological properties.

Undoubtedly the production of higher fungi is a possibility, but its volume will have little effect on protein supplies for the population.

Microscopic fungi have some advantages in comparison with other micro-organisms. Their main advantage is their ability to synthesize enzymes, which hydrolyse hemicellulose, cellulose and partially lignin.

Therefore, with the help of specially selected microscopic fungi we can synthesize protein from cellulose and lignin containing wastes without their preliminary hydrolysis. This ability of microscopic fungi gives the possibility for the extensive annual recycling of the large plant resources of our planet.

The potential of this raw material makes us think that SCP obtained from microscopic fungi will play the main role in the solution of the protein problem in the future. The ability of microscopic fungi to synthesize no more than 1.5-3% of RNA, and to produce wholesome protein and cellulosic enzymes strengthen the possibilities of this trend. The above mentioned ability of the microscopic fungi illustrates their unlimited application in cattle-breeding not only as protein addition, but also as an enzyme preparation which helps to digest rough feed.

The need for complete sterility during production and the more prolonged cultivation (2 - 3 days), we can consider as negative characteristics of microscopic fungi.

Taking into account the positive and negative peculiarities of various micro-organisms their combined cultivation (so- called symbobiotic method) is carried out. We consider this is potentially the most effective way as it gives the best SCP synthesis.

Enrichment of feed directly with microbial protein is another promising trend. The cultivation of yeast, microscopic fungi, and bacteria is carried on simultaneously or consecutively. This trend cannot ensure very good production or a well controlled biosynthesis, but it is easily feasible and doesn't require large expenditures and complex equipment. Nowadays, one widley applied cultivation is that of Spirulina as well as the cultivation of Chlorella and blue green algae. For this we have to solve several problems: cell wall splitting, protein extraction, and their purification from harmfull compounds, etc.

Every separate region should carefully weigh a number of factors such as: the state of cattle-breeding, technical potential, raw material base, etc., while trying to obtain proteins by non-traditional technology. That is why every part of the country needs elaborate models which consider the above-mentioned factors.

In this respect the optimal variants are most complex as each factor should reflect the latest data and the advantages and drawbacks of the given factor. The picture becomes still more complex if we consider these factors isolated from one another. That's why when elaborating optimal regional models we can have different versions.

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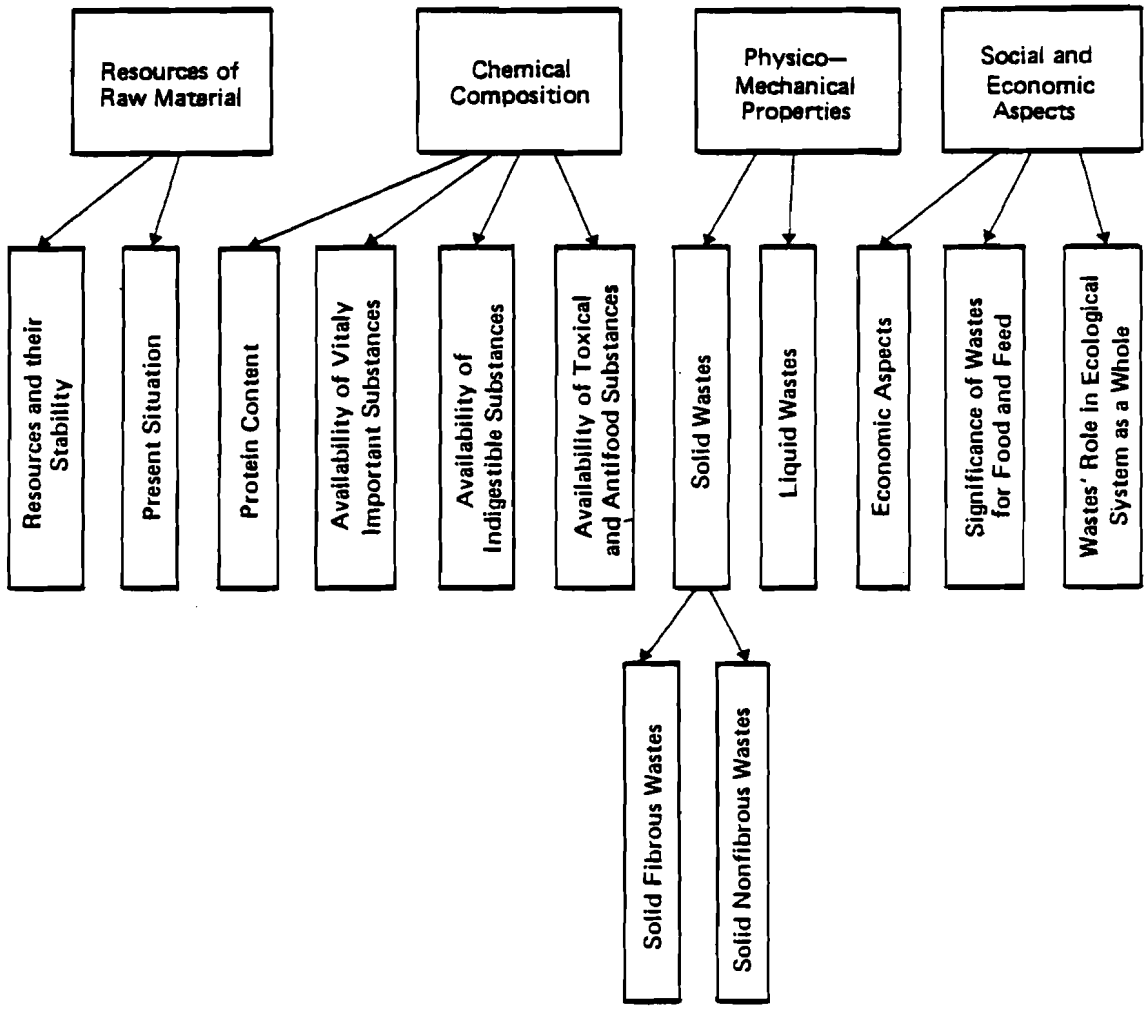
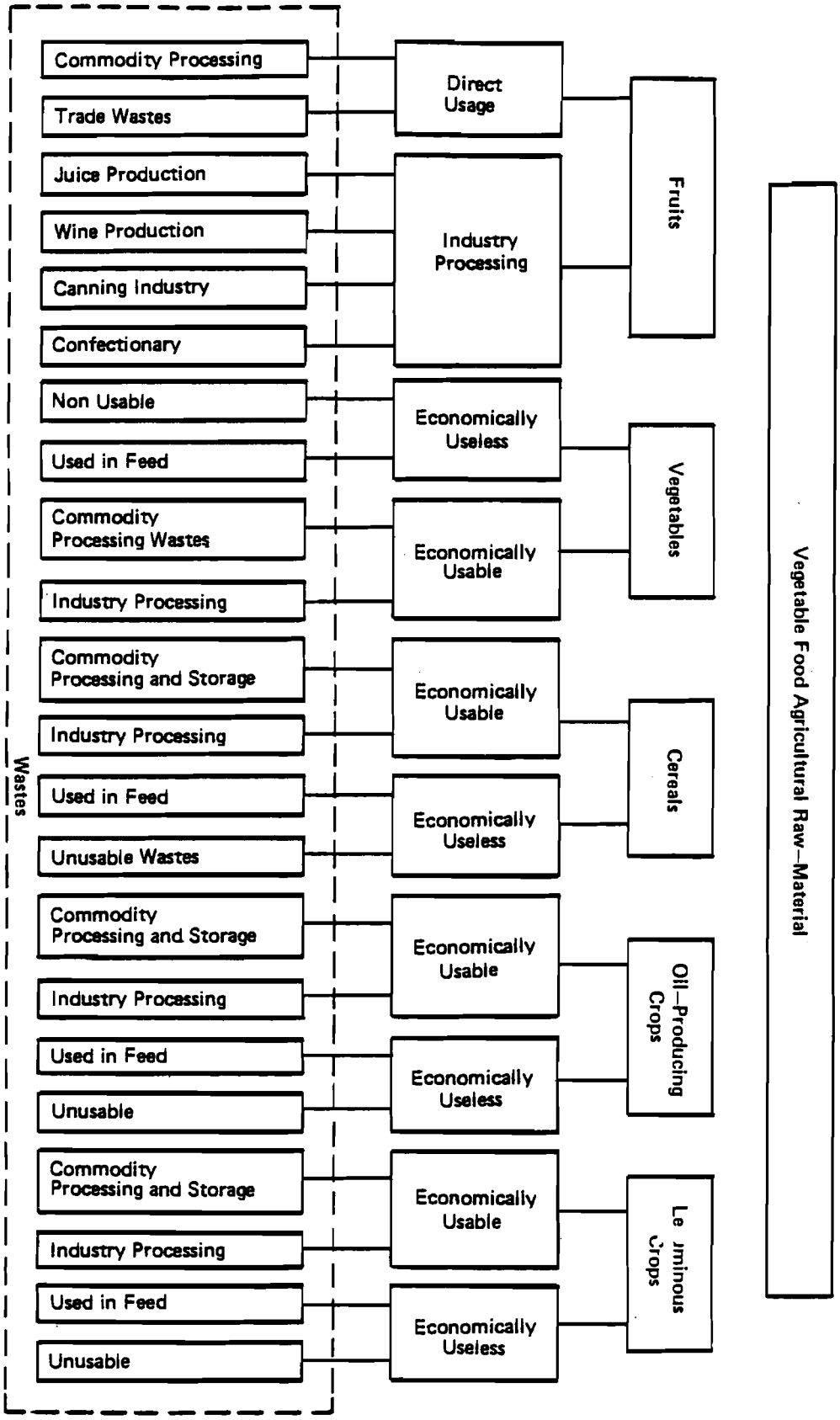


Fig.1 Main factors determining selection and classification of raw material for protein production.


Wastes
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Juice Production
Wine Production
Canning Industry
Confectionary
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Vegetables
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Oil-Producing Crops
Le ɹminous Crops
Vegetable Food Agricultural Raw-Material

Fig.2

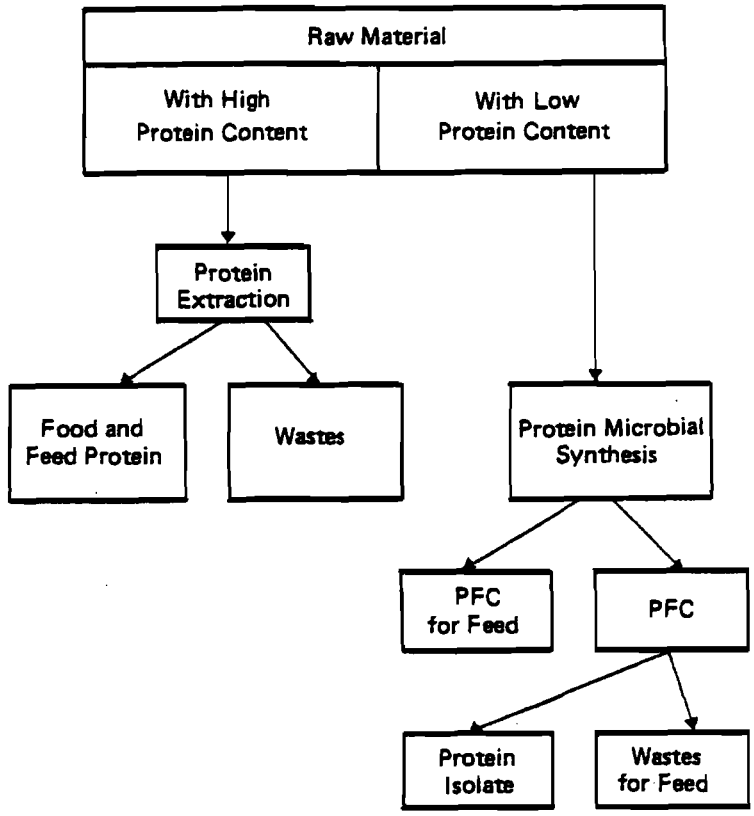

Fig.3 PFC—protein—enzyme concentrate.

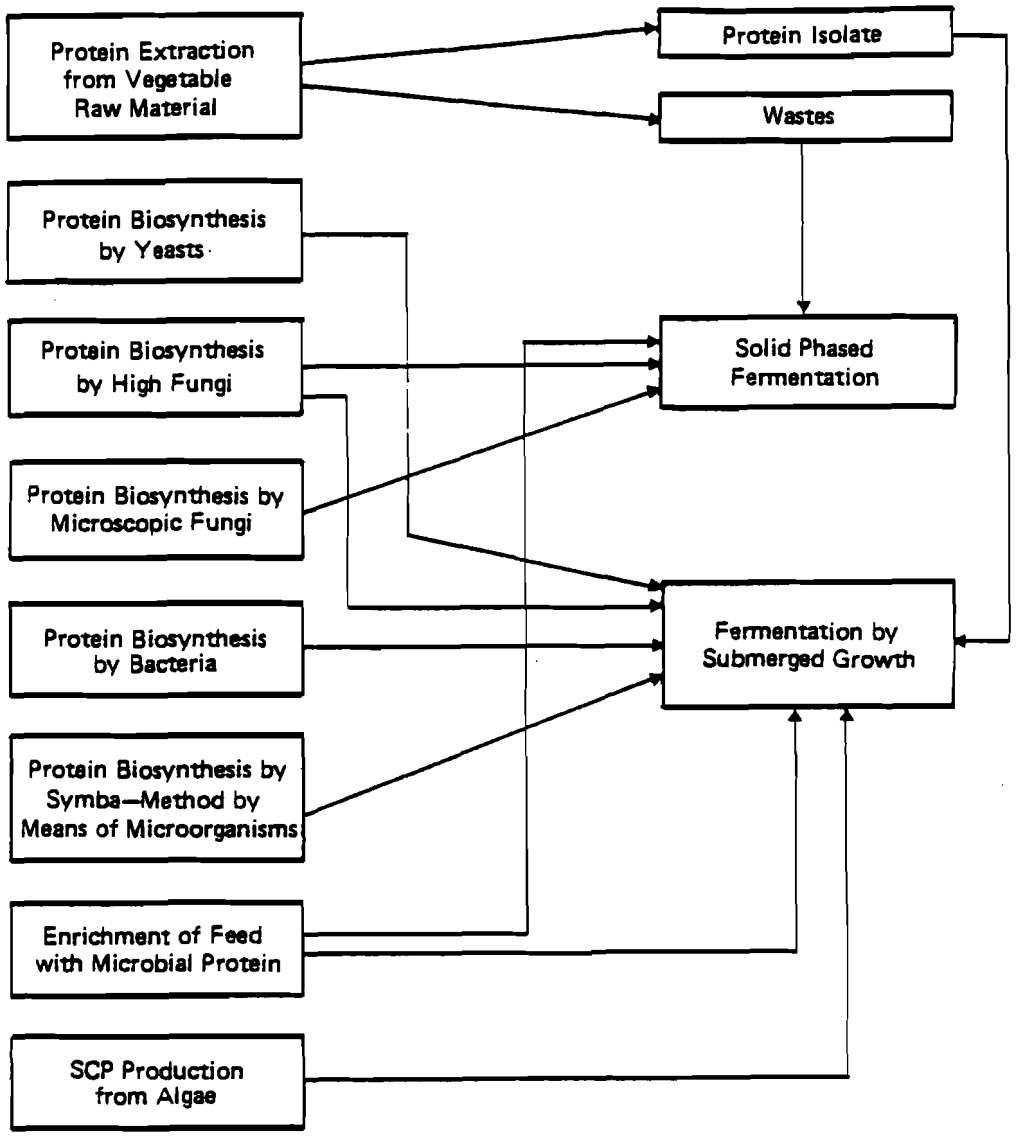

Fig.4 Nontraditional technologies of protein obtaining from vegetable and raw material.

COMPARATIVE BIOENERGETIC EFFICIENCY OF CATTLE PRODUCTION AND BIOTECHNOLOGIES OF PROTEIN PRODUCTION

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1. Introduction

For any manufacturing process an amount of input energy is spent in the engineering system (Ein.), for which we acquire an amount of output energy (Eout.) The efficiency of the system is:

$$\eta = \frac{\text{Eout}}{\text{Ein}} \qquad (1)$$

For a system not connected with production of power conversion of natural power resources the quantity η is always < 1. For bio-engineering systems involving human and solar energy, bio-energetic efficiency may be greater or less than unity.

In Fig. 1 the approximate calculation data of human bio-technological activity are represented ever since the paleolithic age. By spending daily about 3 000 kcal. during the period of the so-called "harvesting" economy a primative man could gather a "harvest" of wild plants, the calorific value of which was a little more than the same 3 000 kcal. Hunting and fishing differ from the previous form of human activity by the growth of power availability per a man brought about by the emergence of specific techniques of obtaining food. By the social division and of labour and the domestication of animals "the energy harvest" collected by a human being was increased.

Proceeding to primitive agriculture (estuary irrigation, shifting agriculture in steppe and hewed timber areas in the middle latitudes) with relatively slight augmentation of the power provision resulted in a considerable increase in the energy efficiency of the exploited system. The power availability and corresponding energy output of the agro-sphere are represented in Fig. 1 by points (D) (fallow and crop rotation and use of organic fertilizers). E (introduction of the foddergrass cultivation and crop seeding at the period of the industrial and agricultural revolutions), F (commencement of fertilizer use) and G (Up-to-date intensive plant-growing). The last point corresponding to H. Huber's data represents Dutch plant growing in 1970: the total annual losses accounted

for 8,3 - 10^3 kcal and the "energy harvest" - $70.1 \cdot 10^{12}$ kcal, these data correspond to $64 \cdot 10^3$ kcal spent every day during the vegetational period per plant specialist and daily energy output in the order of $540 \cdot 10^3$ kcal.

In Fig. 1 the curve AG shows the relative decrease of the agrosystem energy efficiency with increasing power availability.

This is confirmed by the data 3.4 summarized in Fig. 2 (the last points on the curve representing the results obtained in experiments charaterizing "superindustrial" growth of plants.) Accordingly when industrializing agriculture its bioenergetic efficiency decreases. To illustrate this fact we include some extra data.

In 1954 1.05×10^{10} J were spent per ha of maize in 1970 - 3.2×10^{10} J. The power consumption increased by 310%. At the same time the gross yield of grain increased by only 240%. Thus in 1954 1 J energy produced 3.7 J in dry maize, in 1970 - only 2.8 J.

In intensive agriculture the use of fertilizers is the most power-intensive (for cereals and cotton in the USA from 50 to 65% total energy losses). "The energy cost of pesticides" is high as well (from 3 to 10% in different countries). In irrigated lands irrigation is the most power-intensive (at an average more than 60%).

According to the data of foreign statistics in the middle of the 1970s the agriculture of England obtained the annual "energy harvest" of $1116 \cdot 10^9$ MJ. This energy on the market accounted for $138 \cdot 10^9$ MJ. Thus, the biological harvest use coefficient was only 0.12. So small an amount may be explained first of all by the use of more than 70% of the initial energy supplied in plants not directly for human nutrition but for feeding to animals. The energy conversion efficiency by the animals does not exceed on average 6.5%. Consequently, the bio-energetic efficiency is extremely low. According to Huber's data in the middle of the 1970s η (the efficiency) of Dutch plant production is 8.4 and cattle-breeding only 0.14.

The decrease of the energy output efficiency of the agro-system with increasing power availability may be explained by the common laws of biomass growth.

It is common knowledge that in the case of unrestricted growth (absence of environmental resistance) of plant biomass the quantity E which assimilates the power flow grows according to the exponential curve law.

Under real conditions as biomass grows the resistance of the environment strengthens; to overcome it the biomass is forced to spend part of the accumulated energy. Because of this the biomass growth becomes slower.

In a first approximation the biomass growth per unit of time t is supposed to be:

$$\frac{dm}{dt} = rM - pM^2 \qquad (2)$$

where:

r is a coefficient of increment in mass per unit of time;

p is a coefficient of depression (that is mass, which cancels out per unit of time owing to the surrounding pressure).

Let us replace the biomass M by its energy equivalent $E_{out} = EM$ (E is an amount of kcal per unit of mass), time by the input (Ein.) in the agrosystem. The function Ein(t) may be rather complex, it depends on the initial energy level E_o accumulated in seeds, in soil, the way of receiving the solar radiation during

the growing season, time and the volume of plant cultivation. On a first approximation we consider that $E_{in} = E_o + et$ where e is the energy received per unit of time of the vegetative season.

Substituting obtained values E_{in} and E_{out} into the equation (2) and solving it we have:

$$E_{in} = \frac{\gamma \varepsilon E_o [\frac{\gamma}{\varepsilon}(E_{in} - E_o)]}{\gamma \varepsilon + pE_o \{ \exp [\frac{\gamma}{\varepsilon}(E_{in} - E_o)] - 1 \}} \quad (3)$$

Taking account of the equality (I) we obtain the bioenergetic efficiency of the agrosystem:

$$\eta = \frac{E_o}{E_{in}} \cdot \frac{\gamma \varepsilon E_o \exp[\frac{\gamma}{\varepsilon}(E_{in} - E_o)]}{\gamma \varepsilon + pE_o \{ \exp [\frac{\gamma}{\varepsilon}(E_{in} - E_o)] - 1 \}} \quad (4)$$

It is easy to see that the equation (3) is interpreted by the logical curve which has an asymtote $E_{out} \cdot \lim = \frac{\gamma}{p}$ (growth limit, see Fig. 3), the equation (4) - by the hyperlogic curve the limit $r > 0$ while $E_{in} > cs$.

The heometric look of the equations (3) and (4), thus, is similar to the curves Fig. 2.

In practice increase of the "energy harvest" E_{out} in agro-industrialization needs growth energy flows to overcome the resistance of the environment (use of pesticides for example) to maintain its properties (tillage, fertilization).

It is evident that this flow increases when the harvest becomes greater. Pest control is the most typical example. Pest control expenditures increase with growth of the harvest exponentially as pests respond to the harvest growth and to expansion of the forage reserve.

If there is a choice among some artificial biosystems which produce useful products, the efficiency regions of their cultivation are determined by possibilities to provide different energy flows E_{in}. For some biosystems represented in Fig. 3 the following is evident.

If it is possible to provide the power availability in the limit $0 - E_1$, the first biosystem is effective. In the limit $E_1 - E_3$ the second one becomes more effective and in the limit $E_{in} > E_3$ - the third biosystem.

We have made an attempt to evaluate the quantity η and the functioning efficiency of the conventional protein production and the efficiency of diverse manufacturing processes. For this reason the total energy requirements for production of a ton of protein - Ein. - were determined.

$$Ein. = \sum_{1}^{n} E = E_1 + E_2 + E_3 + E_4 + E_5 + E_6 + E_7 + E_8 \quad (5)$$

where:

E - annual energy requirements equivalents for: constructions,
including for irrigation systems (E_1);
machines and devices (E_2);
fertilizers (E_3);
pesticides (E_4);
requirements for plant selection and breeding (E_5);

brain work requirements for the development of the branch (E_6);
direct energy requirements in the biosystem (E_7);
solar energy assimilated in the process of photosynthesis (E_8).

Figure 4 gives the data. The square 1 shows the region of the efficiency of conventional food protein production. These proteins are fed in the form of food mixtures to ruminants (point A), who convert them into animal proteins with an efficiency 6.5 (point B). With increasing energy requirements for providing animals with favorable conditions, the protein yield from the Region II (cattle-breeding) slightly increases (curve BC).

The squares III and IV in Figure 4 represent the region of the efficiency on non-conventional protein production bio-engineering.

The square IV shows the region of the energy efficiency of protein production by micro-biologic techniques. It is obvious that this region has a greater energy requirement than cattle-breeding and is characterized by the greater power requirement. Therefore the solution of the quality micro-biosynthetic food protein problem may meet with considerable obstacles: additional power requirements for improving quality sufficiency produce rather low bio-energetic efficiency of the present bio-engineering.

Essential advantage of the region III over conventional cattle-breeding is in the relatively low "energy cost" with sufficient energy yield. Protein isolate production processes from soya and oil-bearing residues are assumed to be more expensive to a certain extent for improving quality and moreover they remain quite competitive to the region II.

The fractionation of green plants has developed into the most effective manufacturing process. Fig. 5 gives the total energy requirements for diverse methods of production of 1kg protein.

It is obvious that the present level of LPG production provides a high bio-energetic efficiency for this manufacturing process. The cytoplasmic food protein concentrate production being designed at present in the USSR will essentially increase the energy cost of the fractionation, however the latter will remain more effective than conventional cattle-breeding.

Thus, it is true that when increasing the power supply of industrialized cattle-breeding, its effectiveness as a bio-convecter reduces and it is inferior to bio-technologic methods for producing valuable protein. Among these procedures protein extraction from seeds and fractionation of green plants is more effective. The use of photosynthesis in the results is the relatively low energy cost of these bioengineerings.

Research carried out in various countries confirm completely our results. During the past decade techniques and equipment for protein concentrates production from green plants (LPC) have been developed in France, U.S.A., Italy, England, Hungary, Japan, India, New Zealand, Yugoslavia, Czechoslovakia, Poland, Australia, Pakistan, and countries of Latin America. By the end of 1981 in Europe large-scale works with capacities 8-80 t of plant material per hour have been operating: 3 works in France ("France-Luzerne"), 1 in Hungary ("Vepex"), 1 in England ("BOCM Silcock"), 1 in Denmark ("Anhidro"), 1 in Spain ("ARPO-Alfa").

In Italy in 1981 pilot plants with the capacity 3-4 t/h have been tested; in 1982 the construction of the first large scale works begun which uses the process developed by the Institute of Agricultural Industry (Pisa).

In the USSR research first begun at the end of the 1930s but was subsequently discontinued and was recommenced in 1970. Several experimental

plants for the production of juice and protein paste exist and in the town of Mozir (Byelorussia) there is a plant which produces dry protein concentrate and press-cake.

Processing of green plants is as follows: green plant material harvested in the field is transported to the works, unloaded and led to the bin-feeder. A conveyer feeds the raw plant material to the grinders then to the feeder of the first step press. Press cake is fed to the second step press, made friable and then dried or fed directly to the animals.

The second step press-cake moisture is 60-65%, that makes it possible to reduce fuel consumption of the dryer which increases capacity 1.7 - 2.0 times.

The juice obtained is separated from crude matter and cellulose then subjected to heat coagulation in a ejector at 82-85%. Then the coagulum is cooled to 50^0C, fractionated in the filter-press into a paste-like protein concentrate and brown juice.

The paste-like concentrate is dried in the spray centrifugal drier and the dried powder packaged into kraft paper sacks and stored.

Some aspects of the processing elaborated by the Institute are as follows: use of automatic filter-presses, separation of LPG from saponins avoiding the use of evaporators in brown juice treatment and others.

The LPG process in operation in Byelorussia has a capacity of t/h of plant material and will produce per season 1070 t LPG 10300 t of grass meal.

The first lots of LPG were obtained in our Institute from the pilot plant in 1977. The concentrates were separated from saponins and trypsin inhibitor. They contained (%):

protein	56.0	-	62.0
fat	9.2	-	12.9
cellulose	0.8	-	1.8
mineral substances	6.8	-	11.0

Their amino acid composition was similar to that of animal protein, the concentration of unsubstituted amino acids in LPG varied in the range of:

lysine	8.1	-	8.8%
histidine	2.9	-	3.7%
arginine	6.5	-	7.1%
threonine	4.6	-	5.1%
valine	4.9	-	5.8%
methionine	1.3	-	1.62%
isoleucine	4.3	-	4.6%
leucine	8.8	-	9.1%
phenylalanine	5.9	-	6.1%
tryptophan	1.0	-	1.6%.

In addition to the high concentration of valuable protein, 1kg of LPC contained:

carotene	483	-	870 mg
xanthophil	1020	-	1200 mg
tocopherol	200	-	250 mg
vitamin B_2	9	-	13 mg
vitamin B_1	2	-	4 mg

The amino acid composition and the high LPC digestibility (72 - 88%) in vitro made it possible to use them as a source of protein in the diets of chicks, pigs

and calves.

In joint experiments with the All-Union Scientific Institute of Physi-biology and Bio-physics of Animal Nutrition, 50, 75, 100% fish-meal protein and 50% fish-meal, and 50% soybean cake were substituted in broiler rations. Test fodder contained 3.8 - 10.8%.

No essential difference was noted in the live-weight gain between test and control groups of broilers. Average 8-week broilers live weight of the test groups was 1687.3 - 1707.8 g, of the control group - 1717.1g. Fodder consumption for 1kg of weight gain was 2.39 - 2.47 and 2.42kg, respectively.

Protein digestibility of the test rations was in the range 88.2 - 90.2%, control - 88.6 - 89.8%, respectively. Research of the feed protein conversion efficiency into body protein accumulated in the chick's body tissue and liver of the test group was in the same range as in the control group at the start and finish of breeding. No essential difference was noted between broiler groups in slaughter and produce qualities.

The Research Institute of swine-breeding in the town of Poltava conducted a range of successful experiments on using alfalfa LPC in standard diets for pigs - starter SF-19 and SF-24 (growing ration).

Two starter diets were tested: in the first, fish meal and soyabean cake were substituted by LPC; in the second - LPC substituted 50% of dry skim milk. These diets were fed to 42-60 day old piglets.

After 61-105 days of age in the first case, fish meal, soyabean cake and 50% skim milk were substituted by the LPC in the piglets' diet, in the second case skim milk was substituted completely.

Growth and gain of the pigs fed test diets were the same as in the control group obtained with the standard ones.

In the first and second tests where starter diets were fed to pigs the daily average gain was 406g and 390g, in the control group - 412g; in fodder consumption per 1kg of gain - 1.76; 1.83 and 1.74 kg, respectively.

Nitrogen consumption of the test pigs at the starting period was close to the controls (51.8%, control - 53.5%). Test pigs being fed growing rations had nitrogen deposit efficiencies a little higher than in the controls (46.5 and 49.4%, control 41.2%). The amino acid digestibility did not reduce if the amount of the LPC did not exceed 10% supplement to the diets.

The LPC were included as a component into a whole-milk substituent (WMS) in the amounts of 6.3, 12.6 and 18.9%, as skim-milk protein substituent in the amount of 10%, 20% and 30%, respectively. The control calves were fed WMS prepared by the researchers of the All-Union Institute of Animal Husbandry. The test WMS's were not inferior to the control ones in the unsubstituted amino acids content.

The essential amino acids in relation to the non-essential substitute ones were in the range of 0.91 - 0.95, control 0.89. The amount of the consumed WMS's per calf of the test group was about 45 kg, in addition to calves consumed feed-starter and plenty of hay. The daily average live weight, while 10.20 and 30% of skim-milk protein were substituted by the LPC, accounted for 832g, 752g, 724g, the fodder consumption per 1kg of gain in this case accounted for 3.21, 3.44 and 3.62 fodder units, protein consumption accounted for 408.8, 436.1, and 458.3 kg (in the control group the gain accounted for 802g, fodder consumption - 3.31 fodder units and protein 421.7g. The statistical data of the difference between test and control calves live weights are insignificant.

Introduction of the alfalfa LPC into the WMS did not reduce the nitrogen digestibility of the ration. The apparent amino acid digestibility indices of the fodder in the test group varied negligibly and did not depend on the WMS introduction rate. Some lysine digestibility decrease of the test calves was observed (90.0 ... 91.0%, control - 94.1%).

Haematological analysis did not show considerable differences between test and control groups in metabolic and total bio-chemical reactions. Test WMS with 10% dry skim milk substitution by the LPC which had the daily average gain were checked in working conditions. Obtained results were confirmed by the test data. Daily average gain of the calves fed test WMS accounted for 825g, fodder consumption per 1kg of gain was 2.65kg fodder units and 392.7g of digestible protein (in the control group the indices were as follows: 777g, 2.8kg fodder units, 420.8g digestible protein.

The alfalfa press-cake tests were caried out. The silage product after 4-month storage was tested in feeding calves. The press-cake was green, had fruit odour and pH5. The total amount of free organic acids was 1.78%, the lactic acid percentage was 69.3; acetic acid - 30.3. There was no butyric acid in the diet. The dry matter contained: protein - 17.8%, carotene - 134 mg per kg. The ration of the calves consisted of conventional fodders: maize silage - 13.4 kg, wheat straw - 1.2; mixed feeds - 2.7; salt - 0.03; defluorinated phosphate - 0.03.

The test group instead of maize silage and 1.5 kg concentrates was fed 13kg alfalfa press-cake. The calves ate a new diet readily. The daily average gain in the test group was 777g, in the control one - 798g. In the test group 697g digestible protein and 6.9 kg fodder units were consumed per 1kg of gain (in control - 675g and 7.7kg, respectively). The digestibility of the basic nutrients by the test calves was at the control level. The test ration was more economical than the control one.

From the plant material the brown juice had about 16% dry matter containing 14% protein, 35% mineral substances, 40% sugars.

The brown juice as a substrate for yeast was investigated at the yeast factory in the in Zaporozhye. It has been established that brown juice containing 1% and more of reducing substances is a good medium for yeast growth (varieties C).

The yeast yield on the alfalfa brown juice medium was 95.4% of that on the control medium (must made at the works).

Using alfalfa brown juice as a bio-stimulator in feed yeast growth on the hydrolysates and on oil paraffins, postive results were obtained.

The use of brown juice for animal feeding is being investigated.

In 1979, using the same technique of green-chopped alfalfa fractionation (but with the use of two-stepped heat coagulation of plant juice a pilot quantity of alfalfa cytoplasmic protein was produced. The concentrate contained about 85% protein, it was white, odourless and tasteless. Preliminary conclusions of the Institute of Nutrition proved a high biological value of the alfalfa cytoplasmic protein concentrate, showed it to be non-toxic and recommended the possibility of its use as a nutritive material. The leaf protein concentrate contained 85% protein, which was estimated by comparison with soyabean isolate. The investigations showed it was possible to prepare a cytoplasmic isolate with 95% protein content, the quality of which would be considerably higher.

Non-usage of active chemical agents in the ACPC processing is the most important advantage over the soyabean production as it retains the native properties of the raw material and completely excludes toxicity.

Jointly with the Institute of Sanitation and Hygiene of Nutrition, the ACPC biological value was under study.

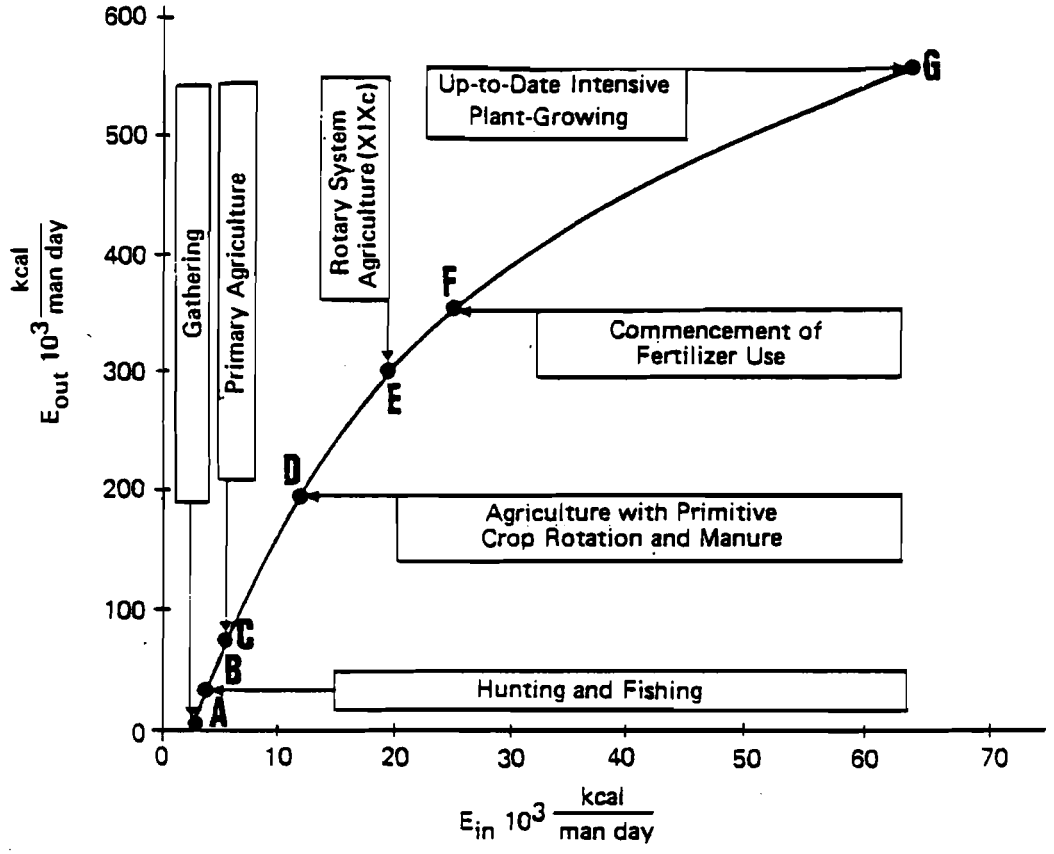
It has been established that the recovered concentrate contained 80% protein. According to the essential amino-acids the concentrate was not inferior to the FAO protein standard, with the exception of the sulphur amino acids (methionine - cystine) the concentration of which were 78.3% of the standard.

The ACPC biological value was determined in the trials on growing rates which were fed synthetic rations. In the control ration casein was used as a protein source, in the test animals, alfalfa protein concentrate. With protein content 10% the digestibility of the test animals was 86.5% of the control ones - 91.3%, the biological value - 83.9 and 88.6% respectively. The protein utilization was rather inferior to the casein control (72.5 and 80.9%). The weight gain per consumed unit (protein efficiency coefficient) in the group was 2.2, in the control one 2.6 - 3. However, it should be noted that in the control diets the surphur amino acids deficiency was not removed. It has been reported that equalizing methionine in the protein concentrate diets to the recommended standards increases the protein efficiency coefficient to 3-5. In this connection it is assumed that removing methionine deficiency in the diets the ACPC nutritive value increases.

In consequence the biological value of the new product from alfalfa juice indicates its good nutritive properties which are close to casein.

Moreover it is advisable to research more deeply the optimization of the amino-acid composition for using this product in bakery and dairy products. The above tests confirm the good nutritional value of the products and suggest their use as follows:

- green protein concentrates in chick, pig and calf rations as an animal protein and soyabean cake substitute
- fodder from the press-cake - in ruminant diets;
- cyctoplasmic protein concentrates as a possible resource in the food industry.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300
200
100
0
$E_{out}\ 10^3 \frac{kcal}{man\ day}$
Gathering
Primary Agriculture
Rotary System Agriculture (XIXc)
Up-to-Date Intensive Plant-Growing
Commencement of Fertilizer Use
Agriculture with Primitive Crop Rotation and Manure
Hunting and Fishing
A
B
C
D
E
F
G
0
10
20
30
40
50
60
70
$E_{in}\ 10^3 \frac{kcal}{man\ day}$

Fig. 1

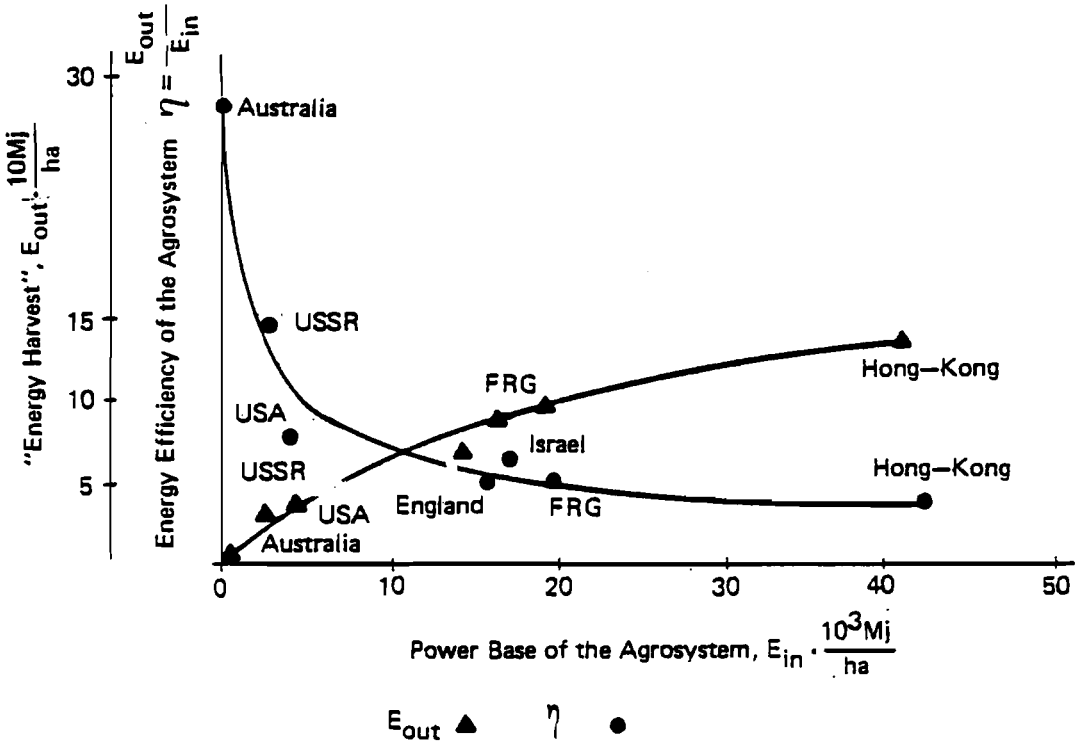

"Energy Harvest", E_{out} $\frac{10Mj}{ha}$
30
15
10
5
$\eta = \frac{E_{out}}{E_{in}}$
Energy Efficiency of the Agrosystem
Australia
USSR
USA
USSR
USA
Australia
England
FRG
Israel
FRG
Hong—Kong
Hong—Kong
0
10
20
30
40
50
Power Base of the Agrosystem, E_{in} $\frac{10^3 Mj}{ha}$
E_{out} ▲ η ●

Fig. 2

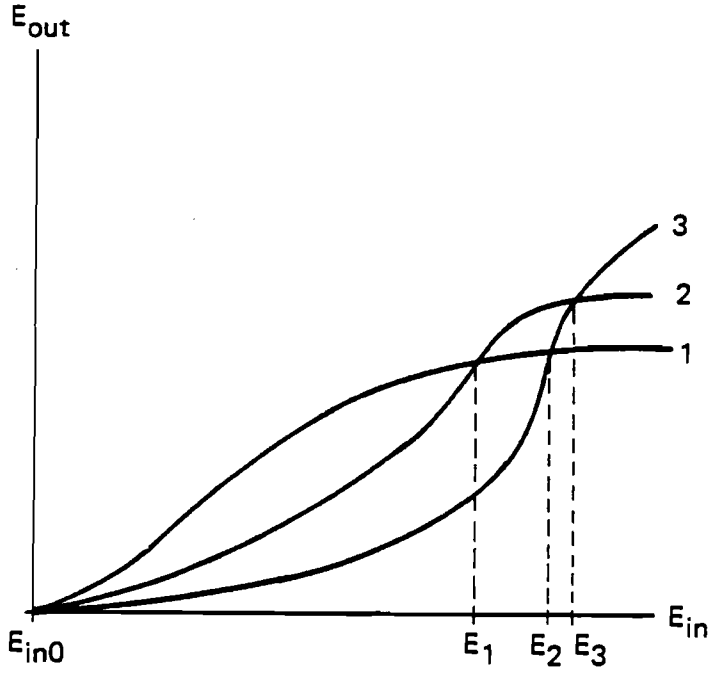

E_{out}
3
2
1
E_{in}
E_{in0}
E_1 E_2 E_3

Fig. 3

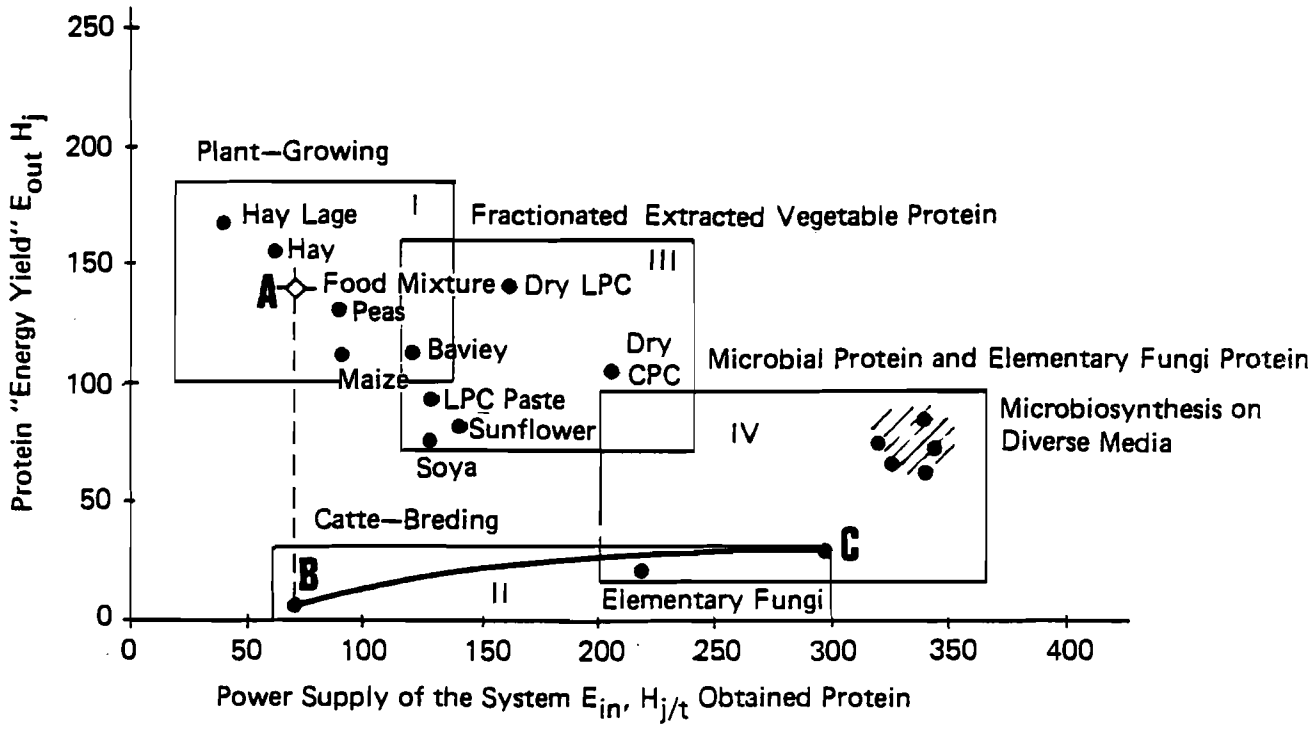
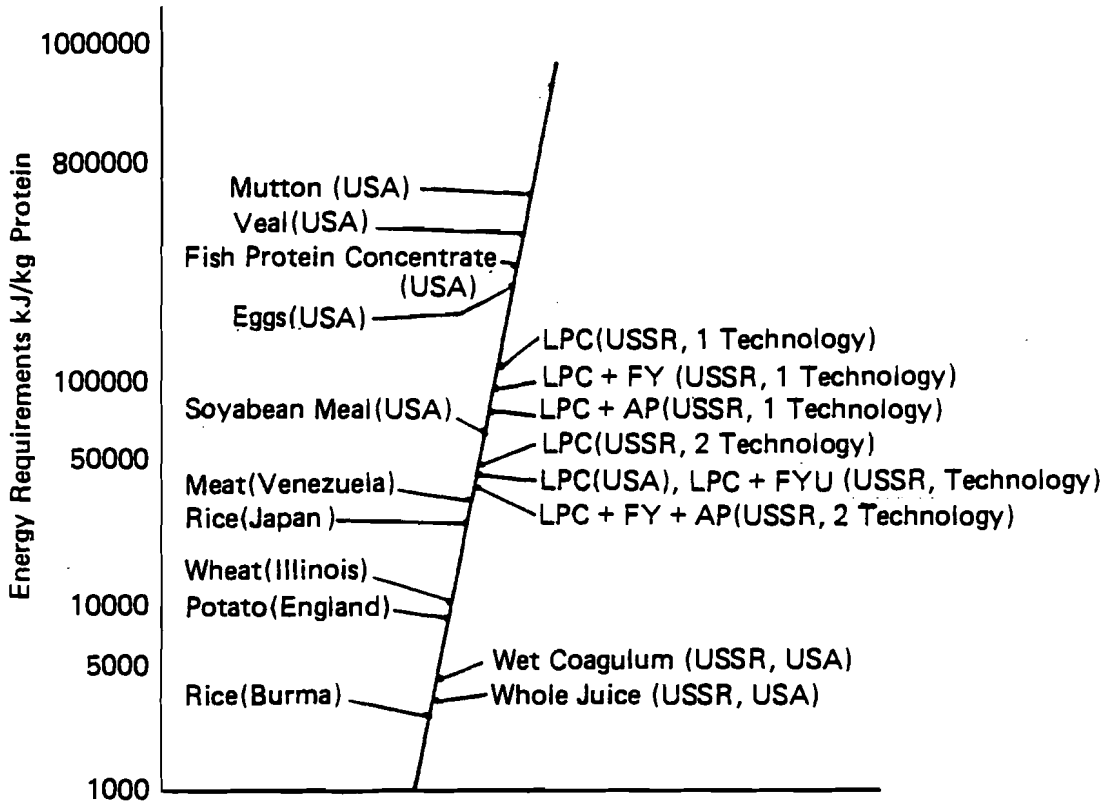

Protein "Energy Yield" E_{out} H_j
250
200
150
100
50
0
Plant—Growing
Hay Lage
Hay
A
Food Mixture
Peas
Maize
Baviey
LPC Paste
Sunflower
Soya
Dry LPC
Dry CPC
I
III
IV
II
Fractionated Extracted Vegetable Protein
Microbial Protein and Elementary Fungi Protein
Microbiosynthesis on Diverse Media
Catte—Breding
B
C
Elementary Fungi
0 50 100 150 200 250 300 350 400
Power Supply of the System E_{in}, $H_{j/t}$ Obtained Protein

Fig. 4

Companson of Energy Requirements for Production of
Various Kinds of Protein



Designation:

LPC—Leaf Protein Concentrate
AP—Animal Protein
A FY—Fodder Yeast
PC—Protein Concentrate

1 Technology of LPC Production (on Spray Drier)
2 Technology (On Fluidized—Bed Drier)

Fig. 5

RICE HULLS AS A POSSIBLE SOURCE OF RAW MATERIAL FOR THE PRODUCTION OF SCP FOR ANIMAL AND HUMAN NUTRITION

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The annual production by photosynthesis of dry vegetable material is in the region of 170 billion tons of which the energy equivalent is 3.2×10^{21} J. This is equal to twenty-three times the energy content of the world's annual production of crude oil. Of these quantities, about one percent is used for food and feedstuff production, and a proportion is also used for energy generation and fiber production. Accordingly, these regenerable sources of carbon represent nearly inexhaustible reserves for bioengineering processes such as the production of high-protein biomasses, fuels for power generation, and raw materials for further product syntheses. Economic utilization of such raw materials for the purposes referred to above is determined largely by their concentration, their incapability of use for other applications, and the development and availability of low-power and technologically simple processes of decomposition and utilization. These particular requirements are essentially satisfied by agricultural waste products from single-crop systems of farming. An example of this is rice wastes in the form of hulls, leaves, and stalks. These constitute the greatest proportion of agricultural wastes in the world and also show considerable rates of increase (Table 1). Ninety-four percent of these wastes are in developing countries.

Table 2 shows a comparison of the composition of rice wastes with other agricultural waste products. What is readily apparent from these data is the high proportion of inorganic components (which is in the neighborhood of seventeen percent) and the small proportion of lignin. The different sorts of rice wastes have a similar composition. The low nutritional value and the structure of rice wastes do not generally allow them to be used for feeding purposes. Some of the parameters determining the nutritive value are compared in Table 3 with the values obtained for alfalfa.

Also, there are virtually no other possible methods of processing such wastes. Consequently, they suggest themselves as raw materials for the conversion of matter through the use of bio-engineering processes.

One of these possible methods is chemical hydrolysis of rice wastes with minimal use of auxiliaries and consumption of power followed by the growth of yeast on the hydrolyzates with the aim of producing protein for farm livestock or directly for human nutrition. Growth of cellulolytic micro-organisms on partially decomposed residues of hydrolysis also provides for additional production of protein-enriched feedstuff for ruminants. It is also readily possible for residues to be used for soil conditioning purposes.

Table 1. Estimated yield (in million tons) of some agriculture waste products and their distribution for the years 1976 and 1985

Waste	World		Developing countries		especially Africa		esp.Southern Africa		esp.Near & Far East	
	1976	1985	1976	1985	1976	1985	1976	1985	1976	1985
Rice wastes	828	1026	770	962	13	17	34	43	385	483
Wheat wastes	579	675	193	244	8	21	23	30	99	122
Cassava wastes	127	158	127	158	47	91	39	62	40	50
Sugarcane wastes	550	714	303	424	18	33	162	217	92	128

Table 2. Composition of agriculture wastes in Cuba

	Cellulosic	Lignin	Pentosan	Ash
Sugarcane bagasse	47.7	20.2	26.5	6.5
Sugarcane pith	46.6	20.2	28.5	2.4
Kemat stem	53.3	17.2	21.5	2.2
Tobacco stem	47.5	16.5	17.8	5.9
Banana stem	23.0	5.3	8.1	9.1
Rice-straw	39.5	4.1	9.6	16.8
Rice-hulls	40.2	4.2	9.5	17.1

Table 3. Nutritive quality of rice-hulls in comparison to alfalfa.

	Rice-hulls	Alfalfa
Digestibility	31%	50%
Protein content	4.2%	17.0%
Digestive-energy	7.76kJ/kg	10.47kJ/kg
Content of crystallinity	45%	32%
Extract without nitrogen	43.5%	40%
Soluble nutritive components	43%	57%
Content of silicates	14.6%	1.5%
P	0.09%	0.23%
S	0.10%	0.30%

Therefore, it is possible to combine the advantage of an increased rate of reaction of chemical hydrolysis with the benefit of high selectivity and minor power requirements of enzymatic hydrolysis.

The kinetics of rice hull hydrolysis were studied using dilute sulfuric acid ($H_2 SO_4$) and reaction temperatures between 80^0C and 100^0 C. The curves of

formation of sugar are shown in Fig. 1.

Pentosans are predominantly decomposed even at acid concentrations of five and ten percent. Decomposition of cellulose is initiated as a competitive reaction. The decomposition of pentosan is shown by the semilog plot in Fig. 2. A number of pentoses and hexoses are produced.

Mild reaction conditions suppressed further decomposition of the sugars produced to reduced organic compounds and furfural, substances that may act as growth inhibitors.

A fast-growing yeast culture was isolated from Cuban citrus wastes and identified as *Candida tropicalis*. It has a wide spectrum of assimilation of sugars and other organic substances (Table 4). This yeast culture exhibited excellent growth on the products of hydrolysis of rice hulls.

Table 4. Oxidative assimilation of sugars and other C-sources with *Candida tropicalis* QML 7601.

Glucose	Xylose
Galactose	L-Arabinose
Sorbose	Salizin
Saccharose	Ethanol
Maltose	soluable starch
Melibiose	Succinic-acid
Cellobiose	Manitol
Trehalose	D-L-lactic acid
Citric acid	

Maximum values obtained from rice hull hydrolyses with 0.2 N H_2SO_4 and H_2SO_4/ HNO_3 mixtures (0.25 N) for the formation of reducing sugars, the yield coefficients of hydrolyzed rice hulls, (Table 5). They were obtained by the design of factorial experiments, and they can be used as starting values of a commercial process. An example (with two variants) of a system having a processing capacity of 10 kilo-tons/year of rice wastes is shown in Table 6. The biomass composition of the culture grown on the hydolyzates is shown in Table 7. The amino acid spectrum can be seen in Table 8. For comparison, the spectra of a number of widely used protein feeding stuffs are also shown. The values are totally acceptable. All essential amino acids are amply represented.

Table 5. Hydrolyis of rice hulls with mixtures of H_2SO_4/ HNO_3 and H_2SO_4, maximum results

Maximum value	mixtures of H_2SO_4/ HNO_3 (0.25 N)	H_2SO_4 (0.1 - 0.2 N)
reduced sugars	0.28g/g rice-hulls	0.32g/g rice-hulls
reduced sugars hydrolised rice-hulls	94.9%	81%

Development of an uncomplicated technology and utilization of the residues of hydrolysis in the manner referred to previously would enable the culture involved and the principle of processing mentioned above to offer an acceptable solution to the problem of producing protein in developing countries with large rice growing areas.

Table 6. Variants of utilization by yeasts of 10.000 tons/a rice-wastes after chemical hydrolysis.

	Variant 1	Variant 2
Acid	H_2SO_4/ HNO_3 = 4:1	H_2SO_4
Concentration of acid	0.25 N	0.2 N
Hydrolysis temperature	121^{0}C	121^{0}C
Hydrolyis time	1hr	1hr
Reduced substances	2800t	3200t
Solid residues	7020t	6050t
Yeast biomass	1250t	1450t
Doubling rate for the biomass	1.1hr	1.2hr

Table 7. Analysis of the biomass of the strain *C. tropicalis* QMC 7601 grown on rice-hull-hydrolysates.

	Content(%)		Content(ppm)
Crude protein	42.5	N	6.8
Nucleic acids	6.0	P	1.6
Fat	0.27	K	2.24
Ash	7.4	Mg	1353
Water	10.2	Ca	384.7
C	42.9	Cu	150
H	6.7	Fe	462.5
Mn	25	Zn	313.2

Concentration of biomass from fermenter effluents is readily possible with the use of well-known basic technological operations such as sedimentation, filtration, or separation up to about 15% SM/kg of fermenter effluent. The selection of a suitable technology is dependent upon local conditions as well as on the form of application of biomass. Use of a liquid substrate and drying is possible for both livestock feeding and production of protein matter for human nutrition.

Biomass need not receive any further treatment in those cases in which it is used as feed for livestock. If it is intended to be used for human nutrition, then this may be possible in the form of whole cells (protein concentrate) after nucleic acid extraction by an efficient and protein-saving method of aqueous alkaline extraction or, else, by isloation of protein from the cellular material (protein isolate) Figure 3. For this, we have developed a process combining the

Table 8. Spectrum of amino-acids from the strain *C. tropicalis* QML 7601 grown on rice-hulls hydrolysates in comparison to other protein feedstuffs (g/gd.s)

	C.tropicalis QML 7601	C.utilis	Soyameal	Fishmeal
Asp	4.6	4.1		5.3
Thr	2.2	2.6	1.6	2.8
Ser	2.1	2.5		2.8
Glu	7.1	4.9	1.6	3.2
Pro	1.7	1.8		4.2
Gly	2.3	2.4		6.0
Ala	3.2	2.8		4.5
Val	2.6	2.2	2.3	2.8
Ile	2.5	2.5	2.1	2.4
Leu	3.6	3.1	3.3	3.7
Tyr	2.4	2.6	1.5	1.8
Phe	2.1	1.9	1.9	2.1
Lys	4.4	3.6	2.8	4.3
His	1.1	1.2	1.2	1.5
Arg	2.6	3.3	4.7	3.9
Cys	0.4	0.4	0.3	0.4
Met	0.8	0.6	0.5	1.2

steps of cell decomposition and protein extraction after nucleic acid extraction.

The operation is performed continuously in a specially designed apparatus at 10 to 14 MPa, T = 160^0 C, t = 10 to 60 seconds, and at an initial pH of 10 to 12. The reaction is stopped by causing the pH value to drop to pH 9 and by cooling (Figure 4). Compared to the conventional method of long-time alkaline decomposition, this short-time high-temperature decomposition process avoids as far as possible such undesirable side reactions as hydrolysis, racemization, and lysinoalanine formation. If necessary, the level of lipid which is not generally objectionable can be reduced by extraction with higher alcohols such as, for example, isopropanol. Suitable control of the process of reaction enables such desirable functional characteristics as water binding power, swelling capacity, and bonding ability to be substantially maintained. In conclusion, it should be pointed out that microbial protein concentrates and isolates cannot, of course, be released for use in human nutrition until results of long-time chemical analyses and medical and biological testing are available.

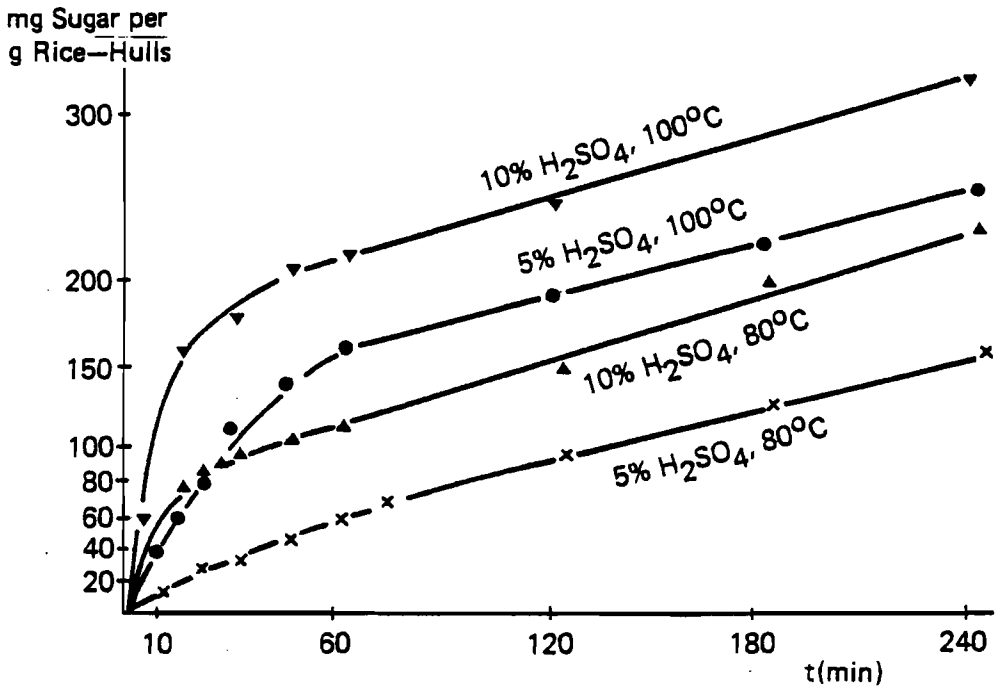

Fig.1 Reducing sugars from chemical hydrolysis of rice—hulls.

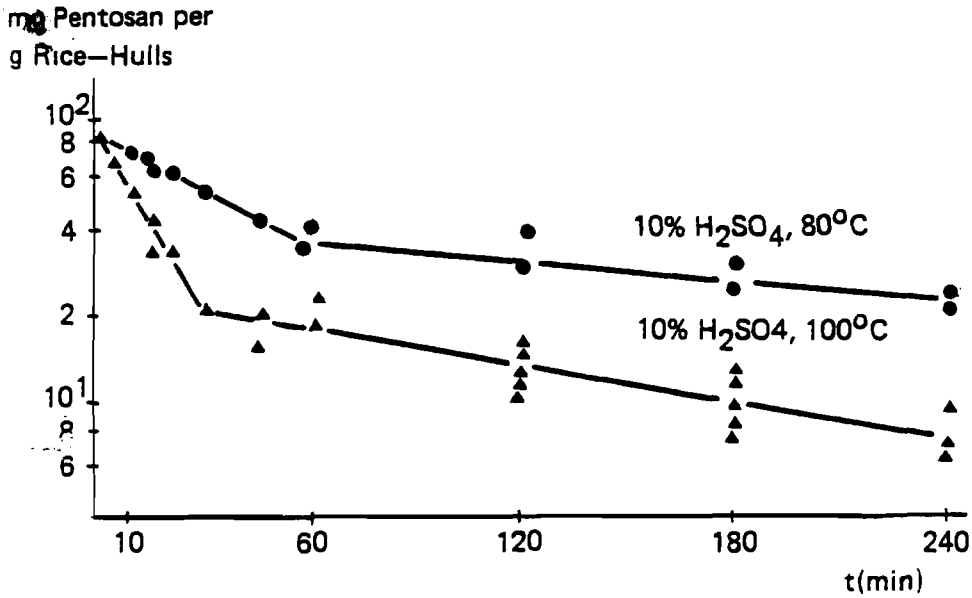

Fig.2 Degradation of pentosan by chemical hydrolysis of rice—hulls.

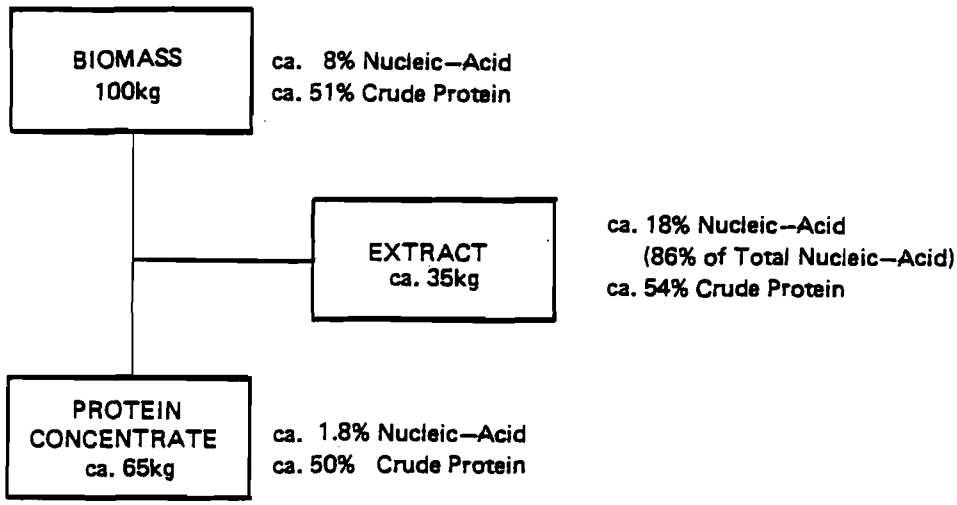

Fig.3 Nucleic—acid distribution from the production of protein concentrate.

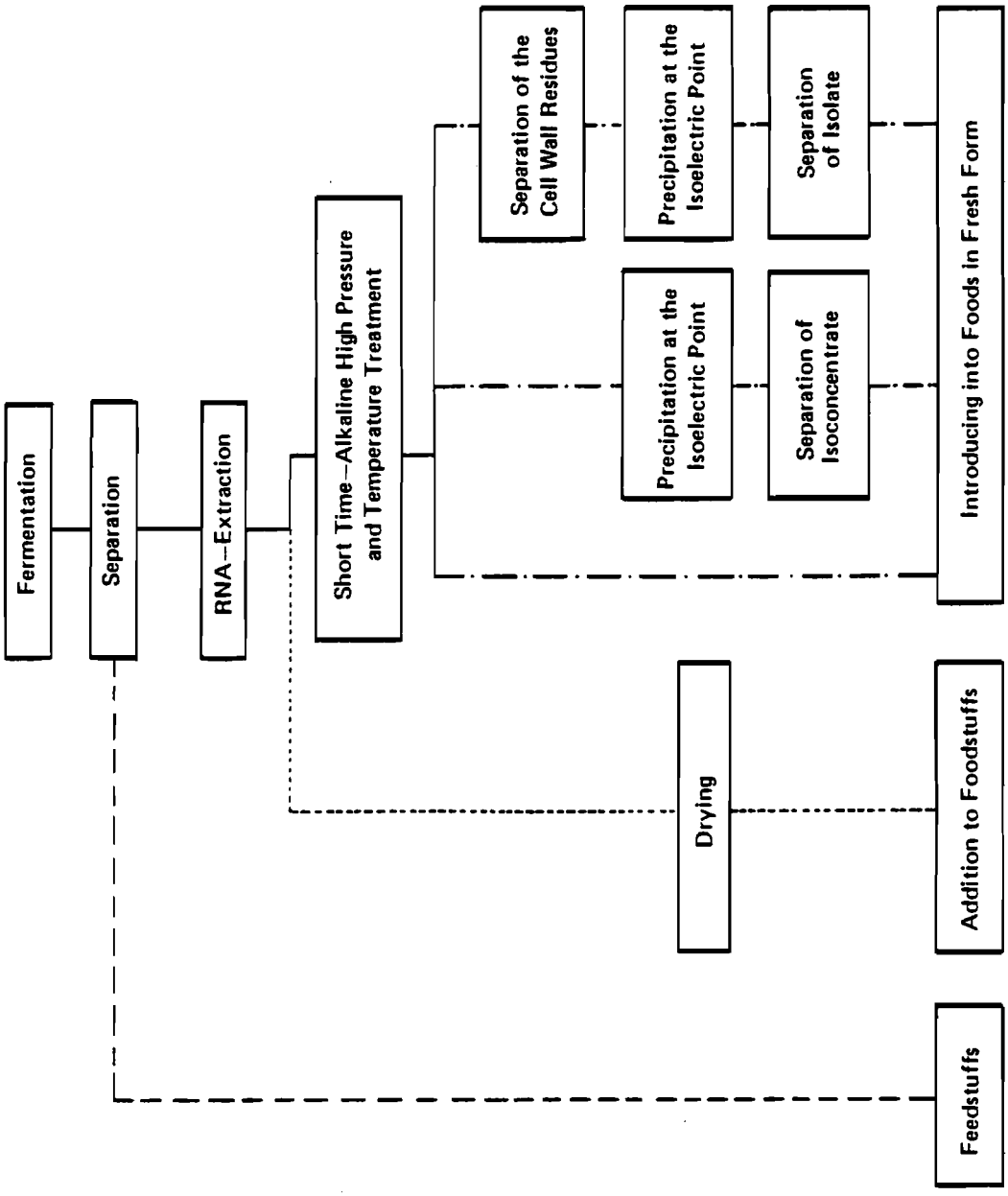

Fig. 4 Fermentation and Downstream Processes

MAIN TRENDS OF PROTEIN PRODUCTION FROM GREEN CROPS

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Taking into account the shortage of protein for food and animal feeding, leaf protein (the isolate of protein concentrates from plant green mass by mechanical fractionation) is of interest. Leguminous plants, for instance, alfalfa *(Medicage)* are known to contain 20% of protein in dry matter. In most countries not capable of cultivating soya, it is possible to obtain upto 10 t/ha of alfalfa dry mass a season, i.e., more protein per hectare than when cultivating soya. According to Stahmann (1974) alfalfa can produce 2700 kg/ha protein a season (Fig. 1), while soyabeans only about 800 kg/ha. Table 1 demonstrates the yield of crop plant dry matter and protein under conditions of the Baltic republics.

Table 1. Yield of dry matter and protein from the cultivation of crops under Baltic conditions. (Grinblats, 1982).

Culture and number of mowings	Yield per 1 ha, tons	
	dry matter	dry protein
Red clover, 2 mowings	5.65	1.06
Red clover, late, 2 mowings	6.29	0.99
Alfalfa, 2 mowings	6.02	1.21
Cocksfood grass, 2 mowings	5.61	0.68
Timothy grass	3.50	0.33
Feed peas	6.54	1.02
Yellow lupin	4.01	0.58
Summer vetch	6.69	1.19
Vetch + oats	6.16	0.88
Maize	9.89	0.93
Feed cabbages	5.18	1.02

As one can see from the table, even under rather severe climatic conditions of the Latvian SSR it is possible to obtain about six tons alfalfa dry mass and almost the same amount of clover *(Trifolium)*.

As it is difficult to cultivate alfalfa seeds in the Baltic republics, a better prospective crop as a protein source is red clover.

On average in the Latvian SSR a continuous crop of green mass from the middle of May till the end of September is ensured providing 5 to 6 tons green

mass per 1 hectare (Grinblats, 1982), including 1.0-1.2 tons protein.

Crops, though containing a large amount of organic substances with a high content of carbohydrates (energy source) and protein, are, however, widely used only as a ruminant feed. In its natural form it is not suitable as food for men or feed for monogastrics due to the high content of indigestible carbohydrates--cellulose and hemicellulose. The content of cellulose in alfalfa mass exceeds 25% (calculated as dry weight), while monogastric rations permit not more than 5-7%.

The idea of green mass fractionation in order to isolate protein concentrates, free of fibre and harmful impurities, appeared long ago. The first publication aimed at the problem was dated 1773 when H.M. Rouell observed coagulation of sediment similar to animal substance, from vegetable juices. More than 40 years ago Slade and Pirie proposed the possibility of using protein coagulates in man's food. The main idea of plant green mass fractionation is given in Figure 2.

In the 1940's systematic investigations were initiated on the process of the green mass fractionation by Pirie (England). At the same time in the USSR Zoubrilin started to obtain a protein-chlorophyllic paste (Zoubrilin, 1943).

A brief history of technologies of protein concentrate production from crops is given in Table 2.

Table 2. Stage of development of work on obtaining protein concentrates from plant green mass

Stage	Year	Authors
Stating of similarity between the basic structural substance of animal and plant cells	1773	H. Rouelle
Formation of the idea of plant protein application in man's food, the first patent on obtaining protein	1926	K.Ereky
Study of the processes of plant green mass fractioning and application of products	1940-1950	N. Pirie,A. Zoubrilin, et. al.
Creating of industrial technology for protein concentrates	1960-1970	N. Pirie, E. Bickhoff, G. Kohler, V. Fomin, J. Novikov, J. Hollo, et al.
Elaboration of the technology for obtaining leaf protein concentrates and use of products of fractionation	1970 upto present	

Now, the agronomy and technology of applying aspects of leaf protein concentrates have been extensively studied and described (Pirie, 1971; 1978; Hollo, et al., 1971; 1972; Kohler et al., 1977).

In many countries (Hungary, France, Spain and others) industrial production of feed protein concentrates has been developed. In the USSR, including the Latvian SSR several industrial installations are in operation. A technological diagram of green mass fractionation is demonstrated in Figure 3. The main stages are:

- harvesting and chopping of green mass at the stage before flowering
- removing of juice in presses
- thermal, chemical or fermetative coagulation of protein
- separation of coagulate by centrifuging or sedimenting
- dehydration or preservation of coagulate and press cake
- utilization of brown juice.

Several technological variants are developed for green mass fractionation to obtain feed leaf protein concentrates. These differ mainly in the final product form and degree of press cake deproteinization, as well as in the level of energy consumption (Table 3) (Novikov, 1982).

Table 3. Energy expenditure on treatment of 1 ton of green mass

Characteristics of technology and products	Total MJ	%	%
Artifical drying of grass (grass flour, pellets)	3494	100	150
Obtaining of dry products and molasses from brown juice	1492	43	62
Obtaining of dry products and yeast (on brown juice)	2411	69	100
Obtaining silage from press cakes, dry protein concentrates and use of brown juice for watering fields	454	13	19
Obtaining silage from press cakes and liquid feed products	203	6	8

Thus, if the highest yield of the protein concentrate is to be reached, it is necessary to separate as much juice as possible (50% of the mass, and more). The juice is optimized as to pH, and the protein is coagulated at 80–90^0. The coagulate is sedimented in decanters, obtaining a paste with 45-55% moisture which is further pelletized and dried in fluidized bed driers upto 8-9% moisture. Press cakes with 63-75% moisture are dried or silaged; the brown juice is evaporated and thus "molasses" is obtained. An approximate balance of the dry matter and protein resulted in the "Proxan" process illustrated in Table 4 (Dolgov et al., 1978).

The yield of the protein concentrate can be raised to 15-20% of green mass protein by a maximum deproteinization of press cakes.

A further increase of the protein yield can be attained by cultivating yeasts on the brown juice (Hollo, 1972), the latter being a complete medium for growing

Table 4. Balance in % of dry matters and protein from the "Proxan" process

Component	Green mass	Protein concentrates	Grass flour	Molasses
Dry matters	100	4	81	15
Protein	100	10	80	10

of a number of micro-organisms. Figure 4 demonstrates the dynamics of the specific growth of the yeast *Hansenula anomala*.

The structure of energy demand when producing protein concentrates is presented in Table 5. The table shows that most of the energy (58%) is consumed by dehydration of press cakes, coagulate and brown juice. That is why the technological variants are of interest which preserve the products of fractionation without involving thermal processes. For instance, the Spanish company "Aproalfa" is silaging press cakes, while the brown juice is used to water the fields.

Table 5. Energy expenditure for obtaining protein concentrate from alfalfa

Operation	Energy Demand, %
Fertilizing	11
Cultivation, harvesting	4
Obtaining of juice	5
Coagulation and sedimentation of protein	22
Dehydration of the products	58

To isolate protein from juice, Stahmann (Stahmann, 1976) suggests that anaerobic fermentation should be used. This direction was further developed by investigations of the August Kirchenstein Institute of Microbiology of the Latvian SSR Academy of Sciences. The fermentative method seems of interest due to the following advantages:

- saving of energy
- inactivation of sapponins, trypsin inhibitor and others without the use of chemicals (acids)
- an increase in the protein quality due to bacterial protein (Table 6),
- preservation of the native structure of protein
- the amount of protein increases on account of bacterial biomass (Figure 5)
- the stability of liquid products (coagulate and brown juice) increases.

For a qualitative spontaneous fermentation of the juice the composition of epiphytic microflora, that get into the juice, is of great importance. The total amount of bacteria and the amount of acid-producing bacteria depend on the type of plant (Table 7) and their growth phase (Table 8). Since leguminous plants for fractionation are gathered at the phase of budding (not more than 3-5% flowers), when the amount of protein in the leaves is at a maximum. It is interesting to note that on the surface of alfalfa at this period there is a maximum amount of acid-producing bacteria, that is very desirable for a speedy process of fermentation. The dynamics of batch spontaneous fermentation process depending on aeration conditions (aerobic variant of shaker flasks) is shown in Figure 6.

Table 6. Comparison of protein concentrates from alfalfa and sugarbeet tops

Method of obtaining concentrates	$N_{tot} \cdot 6.25$, % of d.m.	$\frac{N_{protein}}{N_{total}} \cdot 100$, %	Methionine, % of protein
Thermal from alfalfa	57.1	90.8	1.31
Fermentative from alfalfa	50.6	92.0	1.59
Thermal from sugarbeet tops	45.7	92.8	1.62
Fermentative from sugarbeet tops	47.6	95.2	1.87

Table 7. The amount of epiphytic bacteria in juices of various plants

Juice	Total x 10^6/ml	Acid-Producing		Proteolytic	
		10^6/ml	% of total	x 10^3/ml	% of total
Alfalfa	63.0	0.006	0.01	0.4	0.001
Grass mixture	0.7	0.0002	0.03	3	0.43
Clover	69.5	6.7	9.6	45	0.07
Tops of feed beets	55.4	4.1	7.4	24	0.04
Sugarbeet tops	155.0	39.5	25.5	590	0.38

Table 8. Bacterial flora of alfalfa juice depending on the growth phase of the plants

Growth Phase	Total x 10^6/ml	Acid-Producing		Proteolytic	
		10^3/ml	% of total	x 10^3/ml	% of total
Beginning of vegetation	0.75	23	3.1	70	9.3
Budding	7.6	700	9.2	60	0.8
Blossoming	88.0	20	0.02	380	0.4
End of vegetation	129.8	10	0.01	890	0.7
After-grass	96.9	42	0.04	2761	2.9

Medium aeration brings about a decrease in acid content and an increase in the medium pH by the second day. Thus, it is necessary to maintain anaerobic conditions for fermentative coagulation of protein.

Fermentation of juice can be carried out also in a continuous process (Application for USSR Author's Certificate N 2752320 of Jan. 29, 1980), while dilution rate affects not only the productivity of the system as to acid production (Figure 7 and 8) but also the concentration of both total acids and individual

ones. At a low D (0.03 hr^{1}) acetic acid formation is intensified.

In the experimental system the Institute has installed at the collective farm "Uzvara" in the Bauska region of the Latvian SSR (Figure 9) protein coagulates were obtained in liquid and dried forms, by anaerobic fermentation of green juice from clover, grass mixture and sugarbeet tops. Protein was also obtained from brown juice--microbial biomass (USSR Author's Certificate N 692599) and from processing in straw silos (USSR Author's Certificate N 692601).

The contents of dry protein concentrate from clover is shown in Table 9, 10 and 11.

Table 9. Contents of protein concentrate from green mass of clover

Constituents	Number
Dry matter, %	93.6
Conditional protein (N x 6.25), %	47.6
True protein, %	44.5
Amino nitrogen, mg % to d.m.	15.3
Nucleic acids, %	0.23
Tripsine inhibitor, mg tripsine inhibited by 1 g of dry compound	0.06
Hemolytic index $\frac{\text{ml of 2\% suspens.}}{\text{1 g of d.m. comp.}}$	50.0
Carotine, mg %	10.4
Cellulose, %	5.6

The above mentioned protein concentrate is obtained from sour coagulate (pH 4.0), which was stored at the temperature 15–18^{0} C, for 4 months, then a paste was obtained by centrifuging and the granules obtained dried in a fluidized bed dryer. During storage of the coagulate protein losses were insignificant. The compound contains 44.5% true protein, the limiting amino acid is methionine. The compound is also of value because of carotene and mineral substances, including micro-elements (Beker et al., 1978; 1979).

Making silage from press cakes was studied, too. The dynamics of development of acid-producing bacteria is shown in Figures 10 and 11. On the third day the acid-producing bacteria are the dominating ones in the samples, pH reaches 4.5-4.6. Preservation in a hermetical sealed pack over 4-6 months ensures a high quality silage. The composition of alfalfa silage is shown in Table 12.

Protein concentrates, obtained from the leaf juice by various methods, and the juice itself underwent thorough testing in the feeding of animals (Pirie et al., 1971; Beker et al., 1981). It was noted that biological activity is higher in protein concentrates subjected to acid treatment. It was stated, that in fowl and pig rations, protein concentrates can subsitute 20-30% of protein, traditionally added with soya, oilcakes, fishmeal, yeast and other protein sources.

To illustrate this Table 13 shows the effect of the concentrate upon chickens of up to 30 days of age. The concentrate was extracted from the above mentioned fermented clover juice after prolonged storage.

In Table 13 it is shown that in rations with 20.5% of protein, with a 20% substitution of protein concentrate from clover juice, a stimulated growth of chickens can be observed with an increase of 17.2%; the same effect can be achieved

Table 10. Amino acid contents of protein concentrates from clover (fermented) and alfalfa (non-fermented) (% to protein)

Amino Acids	From Fermented Juice of Clover	Thermally coagulated from alfalfa juice
Lysine	5.6	6.8
Hystidine	1.7	2.4
Arguinine	5.0	6.6
Aspartic acid	7.6	10.6
Threonine	3.3	5.2
Serine	3.7	4.4
Glutamic acid	9.2	12.0
Proline	3.7	4.6
Glycine	4.5	5.6
Alanine	5.5	6.2
Valine	4.2	6.6
Methionine	1.5	2.0
Isoleucine	3.3	5.0
Leucine	7.1	5.4
Thyrosine	3.6	5.6
Phenylalanine	4.2	6.8

Table 11. Mineral contents of protein concentrate from clover after grass

Element	Contents, mg %	Element	Contents, mg %
Na	25.0	Zn	43.3
K	850.0	Cr	2.3
Ca	900.0	Ni	1.1
Mg	200.0	Cd	0.2
Fe	852.0	Co	1.2
Mn	3.4	Pb	2.1
Cu	11.8	Sr	2.7
P	268.7		

at a 30% substitution of protein in the ration, yet at a 40% substitution a negative effect can be observed.

Protein concentrate from clover juice is also a valuable carotene source, the presence of vitamin A in chicken liver testifying to this fact. Biochemical characteristics of blood, such as haemoglobin, blood serum protein, activity of liver xanthine dehydrogenase, etc., in rations enriched with protein concentrate from clover do not essentially differ from those in the control group.

With regard to obtaining protein food concentrates from plant juices, this is the economically the best variant, since convertion of plant proteins in animal organisms is of a very low efficiency.

This can be clearly seen in Table 14, which shows the yield of food protein from various treatments of plant green mass. If the green mass is being dried and hay is fed to milk cows, then from 1 ha at a 24 ton harvest mass with 82% moisture upto 88 kg food protein from milk is obtained with energy expenditure

Table 12. Characteristics of silage from press-cakes

Component	Unit of measure	Number
Dry matter	%	35-37
Protein	%	5.5-7.0
Fats	%	9.9-1.2
Cellulose	%	11-13
Nitrogen-less extract substances	%	10-12
Ash	%	2.5-3.0
Energetic units	unit / 100kg	27-30

Table 13. Biological effectivity of feed, enriched with dry protein concentrate and accumulation of vitamin A in tissue cells of chickens 30 days of age.

No. of group	Ration	Weight gain		Feed expense	Vitamin A in tissues
		g	%	(1g of w.g.)	(in % to 1 and 2 g)
1,2	Positive, control	151.4 ± 4.9	100.0	100.0	100.0
4	20% protein substituted	177.5 ± 5.1	117.2	90.9	121.5
5	30% protein substituted	177.5 ± 4.3	117.2	89.0	150.5
6	40% protein substituted	106.5 ± 5.0	70.3	145.4	133.3
9	1.2g without vitamin A	100.6 ± 4.0	66.4	112.7	30.0
10	4g without vitamin A	170.1 ± 5.6	112.3	96.4	53.3

of upto 918 MJ per 1 kg protein. If the same green mass is being fractionated, press cake silaged, but juice is used to produce milk substitute, the yield of food protein is upto 252 kg/ha, at energy expenditure of about 30 MJ/kg.

Protein, too, can be extracted from the juice, and added to food products. This is described in detail in Pirie's works (N. Pirie, 1978).

Unfortunately, at a differentiated coagulation of protein the yield of food (cytoplastic) fraction is low and, as a rule, does not exceed 20% of juice protein.

The basis of separation of colourless cytoplasmic fraction is the differential heating of the juice: initially upto 50–55^0 C with a subsequent separation of chloroplast fraction by centrifuging, then a secondary heating of the centrifugate upto 85–90^{0}C with a subsequent separation of cytoplasmic fraction of protein. Additional treatment of this fraction gives compounds with a high degree of purity, with protein contents upto 95% dry matter. Such technology is complicated enough for industrial scale production of food proteins. Additional

Table 14. Specific energy expense for obtaining 1kg food protein under Baltic conditions after conversion of fractionating products through animals

Technology	Yield of food protein at 1 ha/kg	Energy expenditure	
		mJ/kg protein	%
Artificial drying of green mass	78...88	816...918	100
Fractionation with obtaining dry products	171...184	129...139	15
Fractionation with obtaining silage from press-cakes and dry protein concentrate	242...252	29...30	3
Same, with obtaining liquid products	252...258	22...26	27

Yield: 24 t mass with 18% of dry matter, in two mowings.
studies in this direction are required.

The information given above leads to the conclusion that isolation of protein food and feed concentrates from crops is a prospective additional way in supplying man with full-value food.

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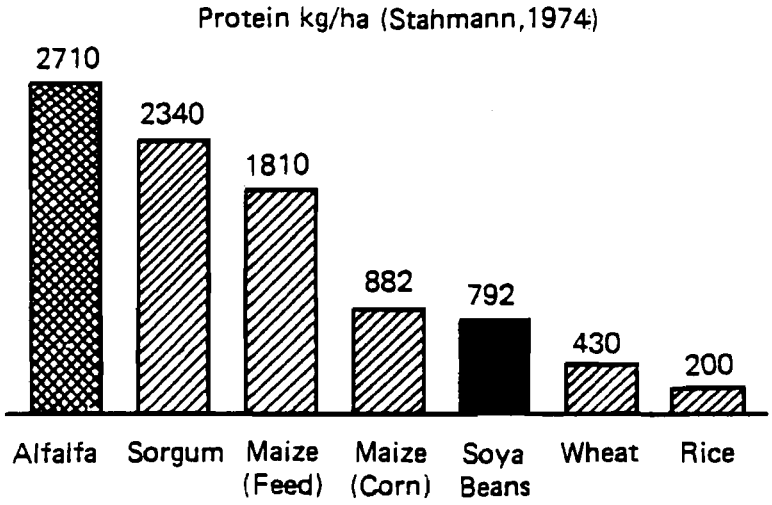


Fig.1 Comparative data on protein obtained from one hectare crops per season.

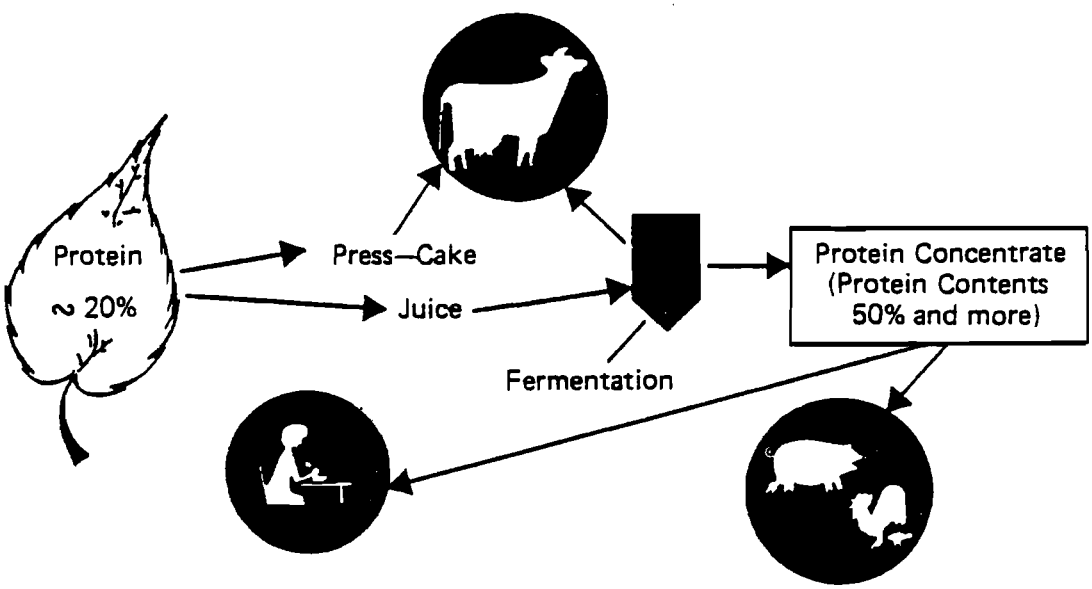
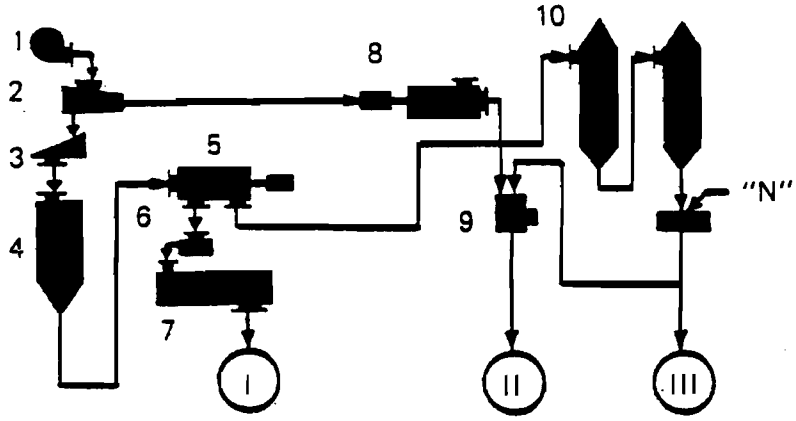
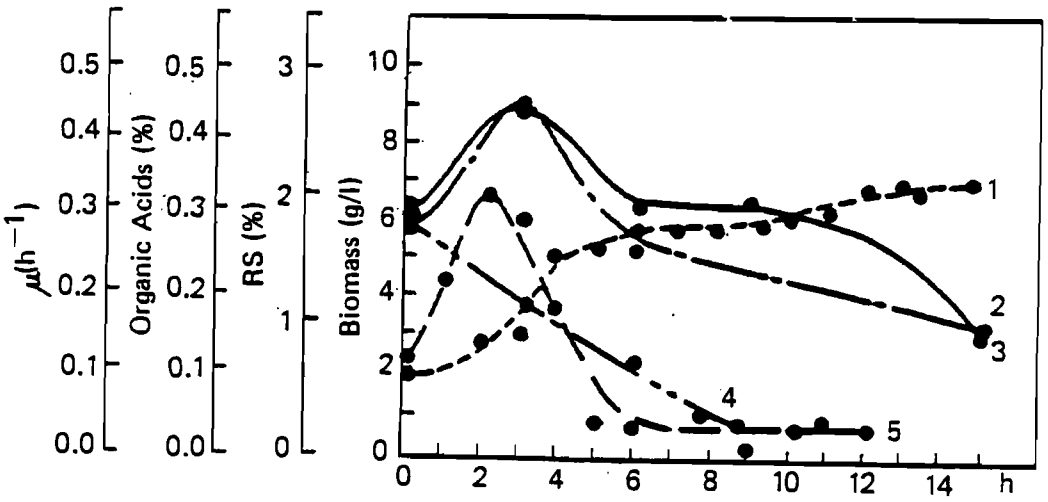


Fig.2 Application of the products of plant green mass fractionating.



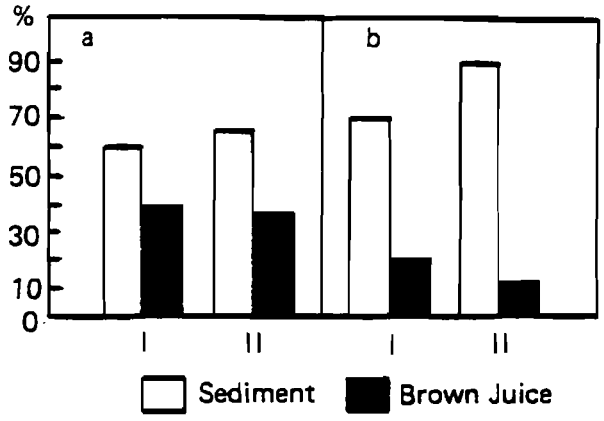
1—Disintegrator, 2—Press, 3—Sieve, 4—Anaerobic Fermentor, 5—Centrifuge-Decanter, 6—Granulator, 7—Drying Chamber, 8—Drying Chamber for Press Cakes, 9—Granulator, 10—Evaporator, 11—Mixer.

Fig.3 The principle technological scheme of plant green mass fractionating.



1—Specific Growth Rate, $\mu(h^{-1})$
2—Organic Acids (%)
3—Reducing Substances (%)
4—Biomass (g/l)

Fig.4 Dynamics of yeast growth on brown juice.



a—Total (Crude) Protein
b—Protein According to Barnstein
I—After Thermal Treatment
II—After Anaerobic Fermentation

Fig.5 Protein Contents in sugar beet top brown juice and coagulate (% of green juice protein)

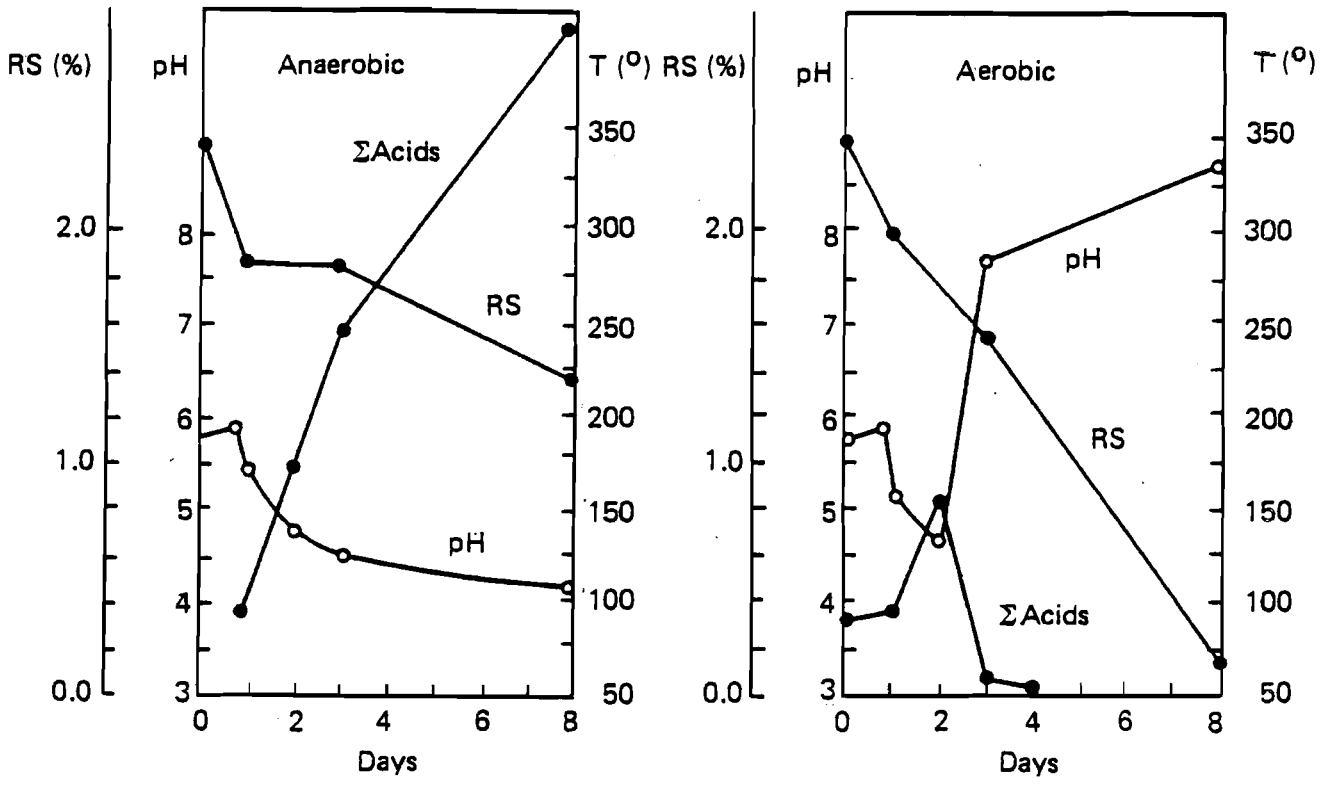


Fig.6 Dynamics of batch spontaneous fermentation process depending on aeration conditions.

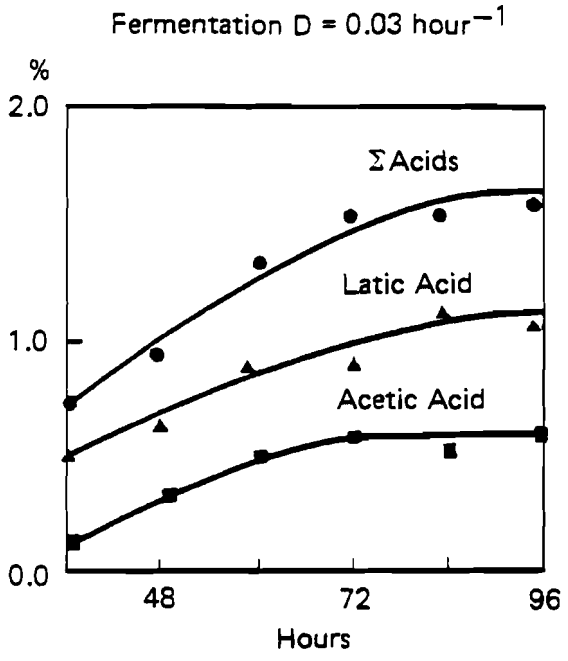


Fig.7 Dynamics of the process of anaerobic fermentation of juice at D = 0.03 h^{-1}.

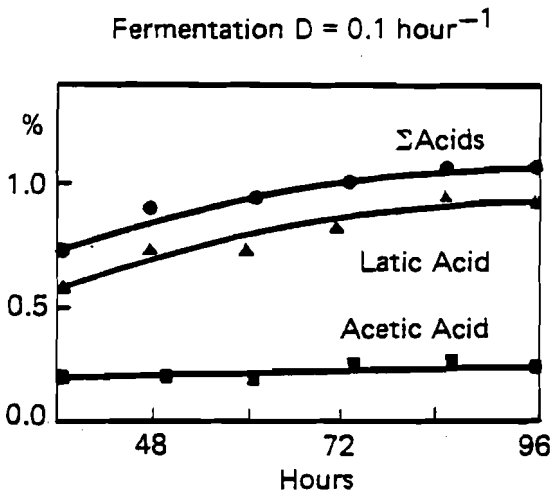
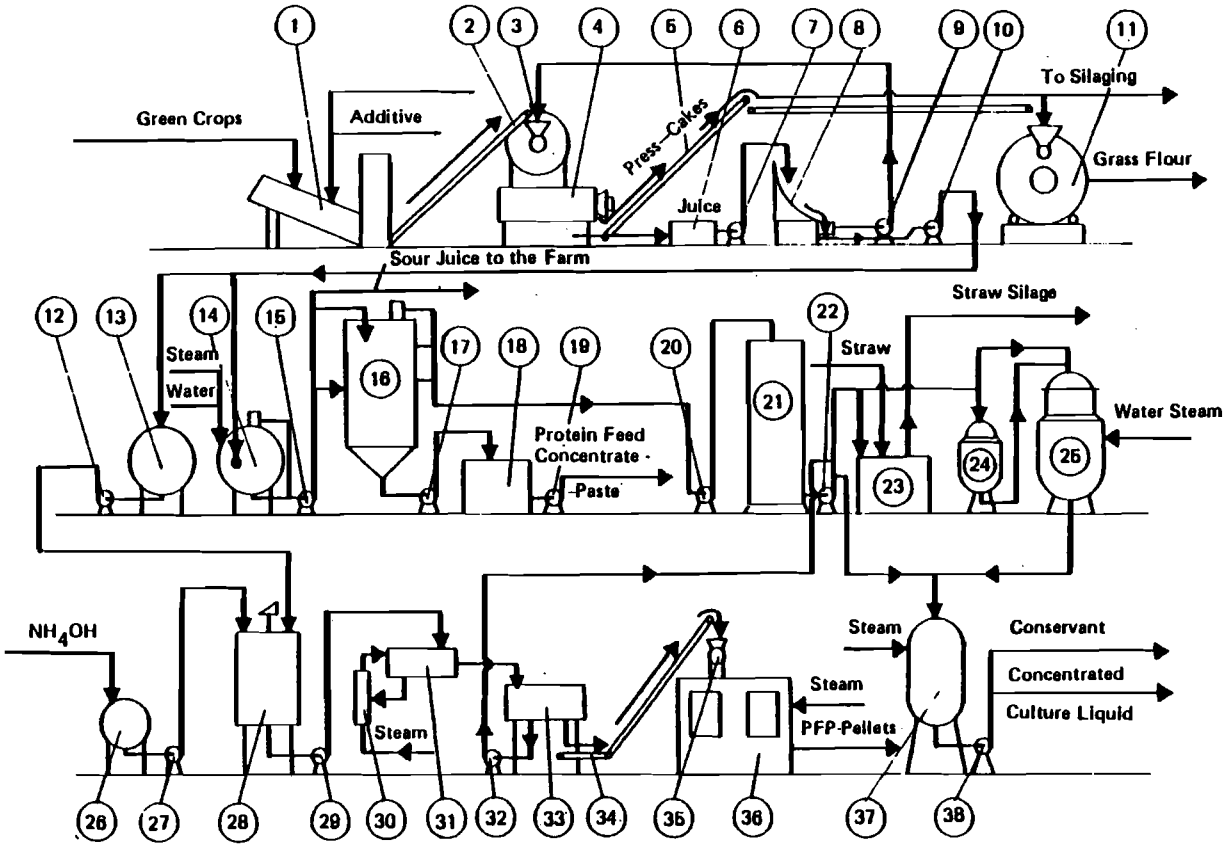


Fig.8 Dynamics of the process of anaerobic fermentation of juice at D = 0.1 h^{-1}.



1–Receiver-Feeder, 2–Conveyer, 3–Shredder–Lacerator, 4–Press, 5–Convayers, 6–Container for Juice, 7–Pump, 8–Sieves, 9–Pump, 10–Pump, 11–Drier ABM-1.5, 12–Pump, 13–Container for the Juice, 14–Anaerobic Fermentor, 15–Pump, 16–Fermenter-Decanter, 17–Pump, 18–Container for the Paste, 19–Pump, 20–Pump, 21–Container for the Sour Juice, 22–Pump, 23–Container for the Straw Silage, 24–Seeds Fermentor, 25–Aerobic Fermentor, 26–Container for Ammonia Water, 27–Pump, 28–Container-Mixer, 29–Pump, 30–Column-Mixer, 31–Heat-Exchanger, 32–Pump, 33–Centrifuge-Decanter, 34–Conveyer, 35–Granulator, 36–Fluized Bed Drier, 37–Evaporator, 38–Pump.

Fig.9 Principle technological scheme of fractionating and bioconversion of plant green mass at the collective farm "Uzvara".

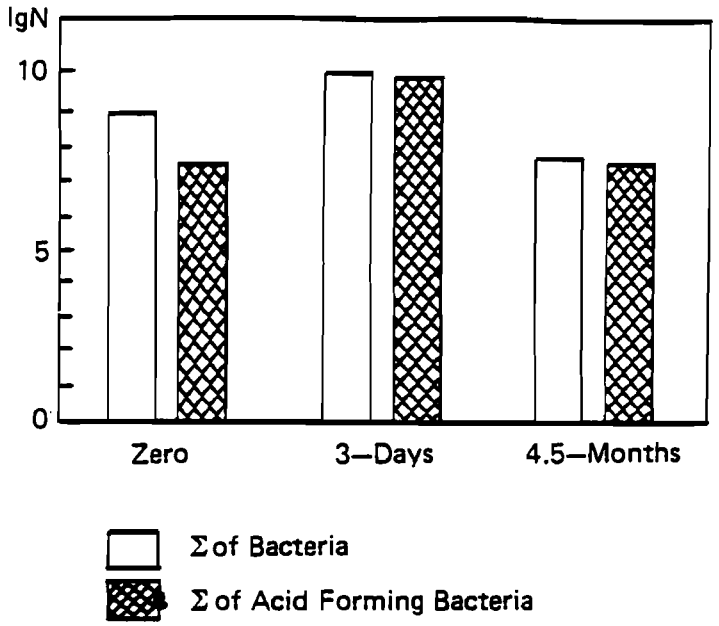


Fig.10 Dynamics of acid—producing bacteria at silaging of press—cakes.

Fig.11 Surface of silage particles and bacterial cells in scanning electron microscope.

WOOD BASED FODDER COMPONENTS

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1. Introduction

One of the most promising non-traditional source for production of protein and energetic components of fodder are the ligno-cellulosic materials (LCM). They are the richest resources of organic matter in the world and their reserves are restored every year by the process of photosynthesis. Their chemical composition is convenient, little differing from the composition of the classical fodder materials. However, because of the higher content of lignin and its bonds with polysaccharide components, it is necessary to treat these materials thermochemically prior to their use as fodder. The intensity of this treatment gives the desired properties of the material in question as well as its applicability. There are a number of possibilities and methods of LCM processing. One scheme is shown in Fig. 1. This is aimed at utilization of the obtained product for the feed and chemical industries and for manufacturing soil conditioner. This scheme was suggested on the basis of the present stage of technical knowledge and possibilities, and the needs of the national economy on the other hand.

It may be seen on the above scheme that it is possible to prepare on the basis of wood or any LCM a standard granulated fodder. The bulk of fodder component is the processed wood material (Fig. 1, pos. 1) being at the same time a carrier of other components and ensuring the stability of the shape of granules. The energetic component is represented by wood molasses (pos. 2) and the protein component by fodder yeast (pos. 3). The essential aminoacids which have to be added to the fodder (pos. 4) can also be prepared by microbiological processing of LCM hydrolysates. The waste product of this process is lignin (pos. 6) which - together with the wood waste of lowest quality unsuitable for hydrolysis - can be used for composting and applied as an organomineral fertilizer (pos. 5) in order to improve particularly the physical properties of soil.

There are many products marked as chemicals which are historically linked with agriculture (production of alcohol, acetic acid) and according to the latest trend their production is becoming based on the plant sources again. A schematic description of the production of the given products is as follows:

2. Direct microbiological treatment

This method requires minimal pretreatment of LCM and provides a material of great importance from the aspects of improving the soil quality. This method is mentioned especially in connection with the possibility of employing it for the utilization of the lignin residue after total hydrolysis for organomineral fertilizers.

3. Thermochemical treatment

It is known that higher plants, especially wood, have a relatively higher content of lignin bonded with polysaccharides, which causes resistance of wood to the microbiological degradation in nature and in digestive tracts of ruminants as well. After thermochemical treatment the lignin-polysaccharide bonds in LCM are loosened and the digestibility of the material is increased several times depending on the intensity of the treatment.

The above mentioned problems have been studied at the Forest Products Research Institute in Bratislava for several years. A number of methods, how to increase the digestibility of LCM, have been tested, such as the combined effect of different catalysts, temperature and moisture content. The treated material was analysed and its digestibility "in vitro" and "in vivo" was tested. Balance tests with selected groups of sheep and bullocks were also performed.

In a series of experiments a special fodder on the basis of treated sawdust was prepared for feeding wild animals in the forest and in the zoo. The results were positive.

Advantages of this LCM treatment can be summarized as follows:

1. Material with low digestibility is transformed into material with high digestibility and nutritive value. By using this method not only the degree of utilization of treated material has been increased, but all parameters of the balance tests have been improved.
2. The treated material is similar to classical fodder as for its origin and chemical composition.
3. Treated material has excellent absorption properties and shape stability after being pressed. Therefore, it is very suitable for preparation of granulated standard fodder with the addition of various feed additives, such as proteins, vitamins, antibiotics and the like.
4. The costs for preparation of the LCM based fodder is adequate and the energy consumption is expected to be below 8 GJ/ton of dry matter.
5. The results of feeding and balance tests with the above materials are promising.

4. Pretreatment of LCM for enzyme hydrolysis

As for the energy consumption and machinery requirement the enzyme hydrolysis represents quite a simple method of directed degradation of wood substance and offers high sugar yields without contamination by the by-products of thermal decomposition. However, application of this method is prevented by the necessity of pretreatment of the material and the high costs for preparation and propagation of enzymes and by the problems with their recovery.

Intensive studies have been carried out on the first part of the problem and there are several pretreatment methods already published, involving different catalysts, different degrees of delignification, etc. In principle, these methods do not differ much from the thermochemical treatment of LCM carried out with the purpose of their increased digestibility. The problems of selection and

propagation of enzyme cultures and their recovery after the enzymatic hydrolysis are beyond the scope of this lecture and will not be considered in detail.

5. Hydrolysis of the LCM

As for the intensity of the conditions of hydrolysis two types of reactions may be distinguished: partial and total hydrolysis. The first method causes depolymerization of hemicelluloses. The obtained prehydrolysate can be processed by evaporation into wood molasses or by dehydratation to 2-furaldehyde. The quality of the molasses obtained by vacuum evaporation of prehydrolysates is approximately equivalent to the quality of sugar cane molasses. Such a prehydrolysate is obtained also in the manufacturing of cellulose or during steaming the wood prior to defibration in the production of fibreboards. The production of wood molasses from these sources depends on economical considerations, especially as for the heat energy demands for its evaporation.

For the total hydrolysis mostly materials from which hemicelluloses were removed by prehydrolysis are used. After hydrolysis of such materials the hydrolysate contain mostly glucose as the product of depolymerization of cellulose. There exist, in principle, two methods of utilization of hydrolysates: evaporation into wood molasses or microbiological treatment. Both methods provide important products for chemical and fodder industry. In general, however, hydrolysates are considered to be more suitable for microbiological processing. Thus the cost for evaporation is saved and the end product obtained has a higher market value. In Table 1 the most important products are listed which are manufactured by fermentation, eventually the manufacture of which begins to be changed from the synthetic method to the microbiological one. Profitability of their production is significantly influenced by the efficiency of manufacture of the glycidic substrate, that is by the technology of hydrolysis. The means that the technology of hydrolysis plays the key role in all considerations concerning the non-traditional methods of LCM utilization.

When comparing different parameters of hydrolysis extreme differences in the values at which the hydrolysis can be carried out, are evident. Due to this fact there are a number of methods and variations of technologies of hydrolysis being developed till now.

It is not the aim of this paper to compare different methods of hydrolysis-technique, but we would like to mention that there is no one method at present which meets all the current requirements. The methods being applied till now in various plants are semicontinuous with all the problems resulting from this. Continuous methods on the development of which many laboratories are actively participating all over the world, have not reached yet such a stage that it would be possible to build them without any risks. To speed up that development, a closer cooperation of interested countries would be necessary.

Table 1. Products of hydrolytic processing of LCM (the world production in 1980 is given in thousand tons in brackets)

Fodder components	Chemicals
Treated LCM	Alcohols:
Wood molasses	• ethanol (700 *)
Fodder yeasts	• butanol
Amino acids	• glycerol
• methionine	
• lysine (40)	Acids:
• glutamine	• acetic acid (1400)
• glycine	• citric acid (750)
• alanine	• lactic acid (50)
• cysteine	• glutamic acid/ monosodium glutamate (300)
• histidine, etc.	
	Aldehydes:
	• 2-furaldehyde (100)

* except from alcohol for production of alcoholic beverages.

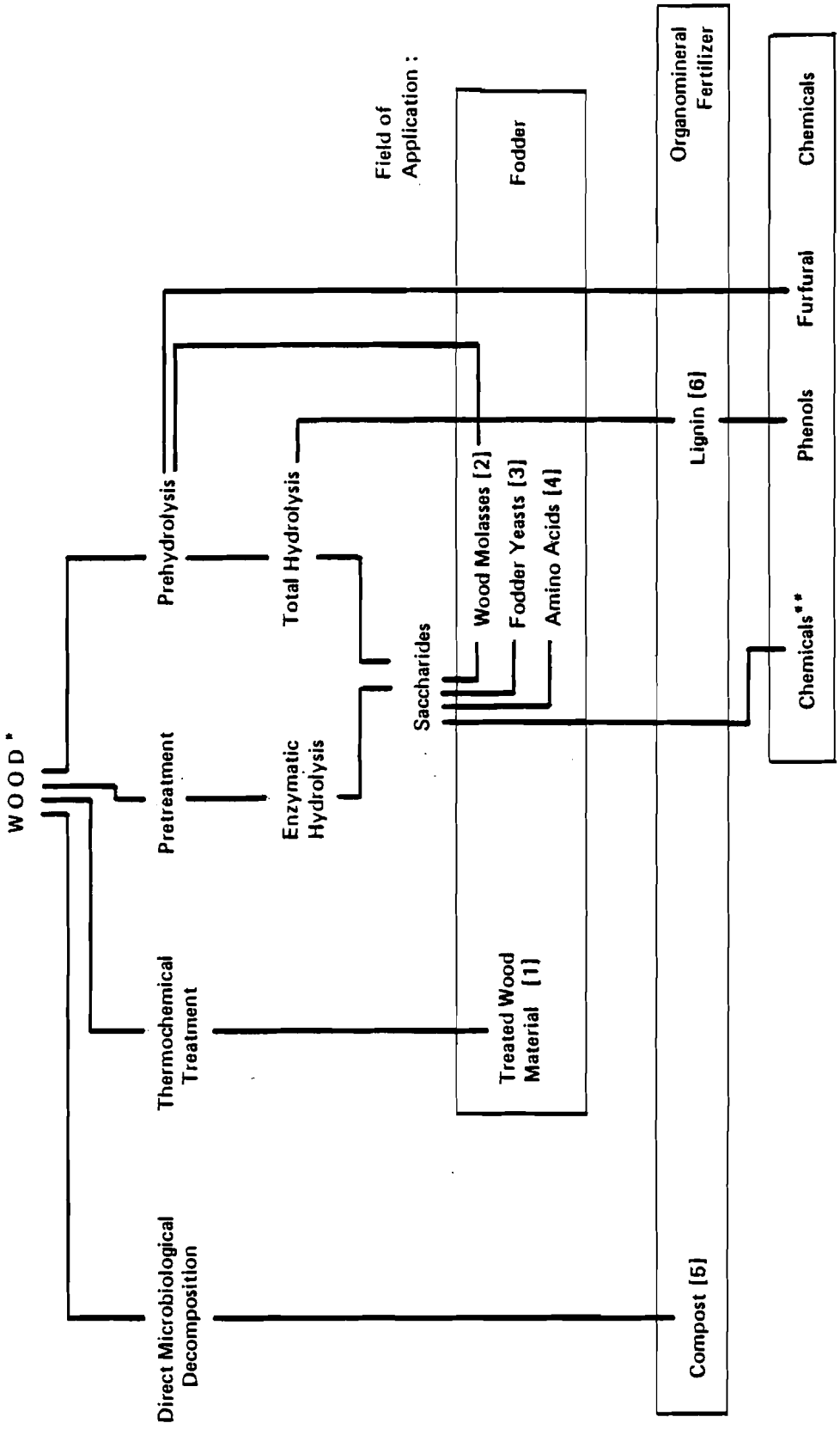

Fig. 1

* In principle, it is valid also for any LCM

** See table 1.

REQUIREMENTS TO THE ENGINEERING SYSTEMS OF BIOCONVERSION OF PLANT SUBSTRATES.

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The problem of securing sufficient food and energy supplies for our planet's present and future population is of great importance to mankind.

In spite of significant intensification of traditional agricultural industry no noticeable increase in efficiency has as yet been observed. In this connection the attention of scientists and economists is attracted to non-traditional methods of food and feed production on the basis of utilization of agricultural and industrial wastes.

New methods of food production do not pursue the aim of full substitution of traditional agricultural production which will undoubtedly remain the main system for solving the food problem.

Moreover, traditional agricultural production will retain its significance for maintaining food supplies and is also the main source base for non-traditional production of food and feed. However, the solution of the problem of non-traditional food production should give us a possibility to solve the food problem to a great extent, particularly to reduce a large part of the protein scarcity.

The processes of microbiological and chemical synthesis, fractionation of green mass of plants, industrial growing of higher fungi, production of pseudosynthetic food-stuffs may be all included as non-traditional production of food and feed.

So-called "synthetic" meat produced in the USA and the artificial soybean sauce produced in Japan are attributed to pseudosynthetic products. Soybean is a base of these products. In the USA 10% meat substitution has been achieved with protein diluents. Overall production of these food-stuffs exceeds 2 milliard dollars in the USA in 1980. At the same time it should be taken into account that soybean is not a characteristic crop for many regions.

The possibility of large-scale protein production by means of chemical synthesis is limited by the modern level of science and technique.

At the present time the industrial growing of higher fungi is rather widely distributed. But fungi are a specific product and are utilized for food in limited quantities that prevents the expansion of the production scale.

Today special attention is attributed to the microbiological production of protein food-stuffs. It is known that this method of protein production does not depend on geographical, climatic and seasonal conditions, does not require a large productive area and utilizes replaceable natural resources.

Arguments in favour of the microbial production of protein and other nutritious compounds are based mainly on the expected economy and assumed scale of production in comparison with protein production from fish flour, plant protein concentrates, etc. Microorganisms as a protein source obtain some advantages in comparison with the most highly productive cattle and plants. The high growth rate and protein production, the ability to utilize different carbon compounds, possibility of rapid selection of productive strains, and the rapidity of microbial protein production are the major advantages in comparison with agricultural production.

The amino acid composition of microbial protein approaches that required for human food (Table 1). Micro-organisms are also rich in vitamins, and other biological active compounds (Table 2). Thus this gives grounds to recommend products of microbial origin not only for feed but also for food.

Table 1. Amino acid content of protein, synthetized by different producers (g/100g protein)

Amino Acid	FAO standard	Fungi	Bacteria	Yeasts	Algae
Leucine	4.8	6.0-9.0	5.5-11.2	7.0-9.0	6.0-8.0
Isoleucine	4.2	3.0-4.0	6.5-6.8	5.0-6.0	3.0-6.0
Valine	4.2	5.0-7.0	4.5	6.3-7.3	4.5-6.5
Lysine	4.2	3.3-7.0	6.5	6.5-6-7	4.9-6.9
Methionine	2.2	2.0-2.8	1.86-2.0	1.2-2.0	1.4
Cysteine	2.0	2.9-3.5	0.6	0.4	0.4-0.8
Phenylalanine	2.8	3.5-5.6	2.9-4.36	4.3-5.3	3.5-5.0
Threonine	2.8	3.0-5.5	4.0-5.4	5.5-6.1	3.9-4.6
Tyrosine	2.8	2.8-3.5	2.5-2.7	4.3	2.6-4.0
Tryptophan	1.4	1.4-2.0	1.2	1.2-1.5	1.4-1.8

Table 2. Vitamin B complex content in micro-organisms and cattle liver (mg/1kg dry compound)

Vitamin	Yeasts	Fungi	Cattle liver
Thiamin	18.2	7.0	4.0
Riboflavin	48.6	55.0	16.0-25.0
Pyridoxine	26.4	25.0	16.5
Nicotinic acid	326.0	440.0	8.0-22.0
Pantothenic acid	100.0	62.0	23.0-51.0
Folic acid	18.0	15.0	3.3-3.8
Biotin	2.7	1.8	-

Usually man uses food that he likes and is accustomed to. The notion of the nutritional value of food is not usually taken into account. It is difficult to expect that the microbial protein will at once and on a large scale substitute for meat in man's diet. Because of that the production of microbial food remains a problem for the future and may not be expected to be applied for some time.

However, the methods of microbial synthesis must play a decisive part in making up the protein scarcity estimated at several million tons.

Fodder yeast production has been developed and explored sufficiently at the present time. The growing of yeast on certain hydrocarbon substrates is realized on an industrial scale in the USSR. This process has been described in detail. It is remarkable that this is a continuous process. In toxicological experiments it has been shown that yeast grown on hydrocarbon substrates is harmless. Yeast is shown to have a high nutritive value by experiments on animals.

The combined processing of wood and agricultural raw materials by means of hydrolysis is an efficient production. At present, the hydrolysis-yeast industry in the USSR is developed in two main directions: obtaining ethyl alcohol by means of fermentation of wood hydrolyzates and other kinds of agricultural wastes, obtaining fodder yeasts by means of growing them directly on hydrolyzates without producing ethyl alcohol.

Recently more attention has been paid to the microscopic fungi as a source of commercial production of protein. Evidently yeasts are very sensitive to the presence of some vitamins in the cultural medium. Yeasts cannot be grown on plant substrates without preliminary hydrolysis. Yeast protein is poor in sulphur-containing amino acids. The cell envelope of yeast is digested with difficulty and requires a preliminary treatment to make its assimilation easy. The high concentration of nucleic acids do not allow yeast to be recommended as food because they are only assimilated in limited amounts.

Fungi as a protein source (by some authors' consideration) have a number of advantages in comparison to yeast. Microscopic fungi have attracted attention for a long time. However, only recently has the production of fungal protein been investigated. Now sufficient data about fungal capability to produce valuable biomass utilizing different substrates have been accumulated. For this purpose, fungi *Aspergillus*, *Fusarium*, *Rhizopus*, *Sporotrichum* and so on are used more often.

The successful solution of the protein problem depends on the availability of cheap raw materials. Recently it has been ascertained that natural resources of oil and gas cannot be regarded as inexhaustible. In connection with this the possibility of increasing fodder protein production using hydrocarbon substrates decreases considerably. Cellulose due to it widespread and high concentration in industrial and agricultural wastes is of great interest as raw material for the microbiological industry.

It is necessary to draw attention to agricultural wastes - straw, leaves and stalks of different plants, stumps of maize, sunflower husks and wastes of the paper industry, low-grade timber, sawdust, etc. - as perspective cellulose-containing raw materials for microbial protein production.

Starch-containing raw materials may be considered also, as in many regions where protein is scarce an abundance of carbohydrates in the form of starch products occurs. It is important to transform a part of such products into protein using the methods of microbial synthesis. This will improve the protein-caloric balance in the countries with protein scarcities.

When choosing raw materials it is necessary to account for its reserves, existence of toxic compounds, it's ability for storage and transportation, traditional ways of utilization, necessity of preliminary treatment before the main technological process (hydrolization, extraction and so on, and the economy of its conversion).

Investigations of the utilization of cellulose from different agricultural wastes and cellulose-paper production wastes have been carried out by scientists in different countries. By many reports it has been shown that the production of fungal protein from cellulose-containing substrates is possilbe and

economically expedient. It has been reported by the Technological Institute of Stockholm that utilization of the approximately 500 thousand tons of cellulose wastes in Sweden annually, could possibly result in a yield of 200 thousand tons of fungus mycelium or about 40 thousand tons of protein a year. The authors point out that the joint cultivation of yeast and fungi (the "Symba" process) is very progressive.

In the USSR extensive investigations have been carried out on the utilization of sunflower husks, straw, corn bran, cotton husks, sugarcane, cotton stalks and the potato processing industry wastes as substrates for cultivating microscopic fungi to produce feed protein. The amount of some wastes is of the order of hundred of thousands or millions of tons. At present these wastes mentioned above are not used rationally.

Peculiarity of chemical structure, physical properties, transportability, localization, seasonal and economic expediency define the choice of alternative methods of processing the raw materials named.

In our opinion the best method is to to divide vegetable wastes of agriculture and industry into three groups:

(1) easily transportable and localized in the same region;

(2) difficult to transport as a rule containing a large amount of moisture;

(3) agricultural wastes which are unprofitable for transportation, especially straw.

Industrial methods of processing cellulose-containing raw materials for the microbiological industry being investigated. Cotton husks, grape pulp and bran are examples. Untransportable wastes which are in general from food processing must be used at or near to the food plants

For processing straw the best methods are those which are easily reproducible at agricultural farms.

The bioconversion processes developed may be divided into two main groups depending upon the of necessity of maintaining aseptic conditions, namely the processes requiring adherence to strict asepsis during the growing stage and those processes which do not require aspectic conditions.

It can be said that the processes for the growing of microscopic fungi must be carried out under aseptic conditions but yeast and some bacteria, possibly due to their high growth rate, do not require such conditions.

Micro-organisms can be grown by submerged growth or deep culture method; or the method of so-called "dry fermentation" known as surface culture. Both methods are aerobic and depend upon a supply of oxygen.

Finally it is worthwhile to mention questions of preliminary processing of plant substrates preceeding microbial growth which are used to increase the degree of bioconversion by microscopic fungi or bacteria or are used to obtain free sugar for growing yeasts.

The processes for the cultivation of the micro-organism can be carried out by both continuous and batch methods following the preliminary substrate processing.

The analysis of the investigations carried out to produce microbial protein from industrial and agricultural wastes shows that most investigations have been carried out in the developed countries. These investigations were the basis on which the complex methods of commercial protein production by submerged culture have been proposed. (The technological realization of such methods requires sufficient financial and developed commercial organization. In the

countries with protein scarcity such a combination of these conditions do not often occur). Nevertheless it should be noted that it is the only possible method of the bioconversion process for a number of cellulose- and starch-containing wastes. In particular this is true when we speak about liquid or moist wastes of some branches of the food industry. It is not necessary to mention in detail the equipment of the submerged culture process in this report as information on these processes is widespread.

The technological processes as a whole may vary at the stage of preparation of the culture broth when native raw materials, extracts or hydrolyzates of plants may be used. Several methods of plant material preparation may be used, e.g., crushing, extraction, hydrolysis. Sterilization of components may be fulfilled by both batch or continuous methods.

The raw materials extracted may be used as the basis for the preparation of culture media for growing the micro-organisms. The residues may be also hydrolyzed to hydrolysates for growing micro-organisms or may be utilized as substrates for surface culture to obtain additional protein product.

The fermentation unit depends on the method of production and may be either a sealed or an open system.

Production of the finished protein products from culture broths may be carried out either by separating the biomass and subsequent drying, or by homogenizing the culture broth followed by drying. By the first method the utilization of the liquid phase acquires significance, by the second version it is necessary to concentrate the culture broth as the content of the dry compounds is at a low level as a rule.

Economic analysis of the submerged culture process for microbial protein production shows that important factors for the organization of profitable production are the following: the cost of raw materials, turnround of productive equipment, and the capacity of the plant.

Continuous technological processes are the most progressive from the economic point of view. As has been noted above, continuous processes are used to obtain fodder yeasts on a commercial scale. This method gives a possibility to increase the profitability of the plant significantly.

Production based on continuous processes puts a number of problems before researchers. In the separation and selection of strains of micro-organisms it is necessary to find cultures with a short life-cycle, that can grow under extreme conditions. These properties prevent them from contamination during growth to a certain extent.

In relation to technical aspects of continuous production the process of continuous hydrolysis of cellulose-containing materials must be considered. At present the equipment for the fermentation hydrolysis of raw materials has not been developed and this stage is a decisive one for some technological processes.

Although microbial surface culture (dry fermentation) has the disadvantage of slow growth on solid substrates, and less protein content in the finished product, since it contains residual substrate, the method has its own advantages in comparison with the method of submerged culture. They are

(1) the equipment is simpler;

(2) the product's drying process does not require a large power input expense as the moisture content of the product before drying is about 35-45%;

(3) the yield of the product from the same volume is large in comparison with the yield from submerged culture.

These factors suggest a considerable economic advantage for the method.

However, it is difficult to imagine that the large-scale production of protein may be oriented to the more traditional technological methods of surface culture based on the use of vessels for microbial growth in a layer of 1.5-2cm.

Mechanization and automation of the processes of surface culture have attracted the attention of researchers and engineers for a long time. Both abroad and in our country mechanized growing chambers of different constructions have been proposed. The main principles of these constructions are the following: vessels and carrier with the culture moving at a given rate through a chamber or a tunnel, with moisture and temperature conditions controlled, in addition the vessel's movement may be direct or multilayer; the culture is grown in a layer of 200mm or greater in a drum (the plant of Valershtein, USA) rolling slowly to obtain more optimal aeration of the culture and to reject gaseous products of metabolism; the culture is grown in a layer of 300mm or greater in a vertical growing plant with aeration and at a given time interval the culture passes from the upper section of the plant to the lower one, this gives a possibility to carry out a semi-continuous process when utilizing several sections; the process of culture growth is carried out in a rotating plant in which it is possible to obtain optimal conditions for a given stage of the micro-organisms growth in certain compartments of the plant.

Discussing these types of equipment for surface culture it may be noticed that numerous attempts to develop mechanized plants on the basis of utilizing these vessels have not given good results as they are found to be metallocapacitive, complex by construction and low in productivity.

A mechanized plant is operating in the USSR at a factory of the microbiological industry for protein production. It does not seem to be satisfactory as it is combersome, has a low productivity and microbial growth is limited significantly by low aeration.

The mechanized sectional plant for microbial culture in a medium layer of 300mm and higher at volume aeration has its own merits and demerits.

Without giving a detailed description of other types of plants it is necessary to note that the engineering decision of microbial surface culture by mechanized methods has not been adopted as a practical method anywhere in the world. In connection with this the development of simple constructions with high productivity is of great importance for introducing into operation the method of bioconversion mentioned above.

A comparison of technical-economic characters of protein production on the basis of cellulose-containing wastes of agriculture and industry by the methods of surface and submerged culture has shown the advantage of the method of surface culture as the cost price of the product obtained is by 1.5-2 times lower than it is by the submerged culture.

An investigation of the profitability of the microbial protein production from the point of view of productivity doesn't yet give a single valid answer. Only large-scale production of not less than 10 thousand tons a year may be profitable in the opinion of the authorities. By our calculation the cost price of the product is 1.5 times higher when the productivity of the plant is 10 thousand tons/year than when the productivity of the plant is 350 thousand tons/year.

At the same time the increase of productivity involves a number of serious problems, namely, precise and uninterrupted provision of raw materials, power, manpower, etc. It's enough to say that consumption of raw materials in a day

will be 1500 tons for a plant's productivity of 350 thousand tons of protein in a year.

So the decision of problems of large-scale protein production on cellulose-containing raw materials is only possible by integrated investigations of different specialists. Only the combined efforts of biologists, engineer-technologists, constructors, economists and agriculture workers can finally lead to the development of optimal technological processes the realization of which will have an economic effect in industry.

For implementing the industrial protein production on cellulose in the future, some methods of bio-transformation of such plant raw materials as straw have been just introduced at Sovkhozes and Kolkhozes in the USSR. The method of preliminary fermentation hydrolysis of straw followed by yeast culture on hydrolyzates developed at the research institute "VNII biotechnique" is extensively developed and may be introduced at any animal feed supply centre. The process is carried out with routine equipment for fodder mills, the main technological unit is a feed blender in which both the process of fermentation hydrolysis and growing of yeasts are carried out. Straw is preliminary cut very small, up to 1.5-3cm, the charge is accomplished by a scraper conveyer. Hydrolysis of straw and growing of yeasts are carried out at a moisture content of 60-70%, which is a semi-dry system. During such processing the protein content increases from 2-3% up to 10-12%, the protein digestibility is 65%. Feed obtained has a a nutritive value 3-4 times higher than native straw according to authoritive estimations.

At present the method developed has been introduced in practice in the USSR. It should be taken into consideration that collective farms and fodder supply centres must be provided with standard inoculum the production of which should be carried out only at specialized stations that can ensure its quality. It is essential that scientific investigations of protein production by methods of microbial synthesis are carried out together with estimations of the safety and nutrient value of new feed and food products obtained. However, as is shown by numerous data the more extensive investigations are stimulated by economic and technological reasons.

Feed and food products obtained commercially and especially at collective farms by means of microbial synthesis must be absolutely safe. This requires a careful study of any changes in the micro-organism's characteristics in relation to conditions of growth and the nutrient medium. So it is necessary even at the early stage to follow the next criteria:

- absence of toxigenesis
- variability limited
- intensive growth on substrates selected
- high protein content of good nutrient value i.e. including all essential amino acids in quantity and relations near to FAO standards
- low lucleic acid content
- high digestibility, that to a certain degree depends on cell wall composition
- contamination stability

And when considering food protein products it is obvious that taste, smell and texture are also important.

Thus, the combined solution of problems of microbial protein production on the basis of cellulose-containing wastes from agriculture and industry could become a means of reducing the protein scarcity which will increases considerably in the near future.

NEW TECHNIQUES FOR THE IMPROVEMENT OF INDUSTRIAL STRAINS OF MICRO-ORGANISMS

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Micro-organisms have economic importance for a multitude of biotechnological procedures including especially those which may solve the problem of the shortage of protein feed and food components.

Genetic methods play an important role in the development of such micro-organisms. Microbial strain improvement programs in the past have been based essentially on random mutation and selection rather than on more rational methods founded on an understanding of genetics and biochemistry of the micro-organism. Over the past few years several novel techniques for genetic manipulation have been sucessfully applied for the construction of industrially important strains. They include directed screening, induced protoplast fusion and recombinant DNA technology.

Directed screening procedures used for selecting regulatory "high yield" mutants are presumably based on selection of antimetabolite resistant mutants which no longer are subject to feedback repression or catabolite repression resulting in overproduction of particular metabolites. "High yield" mutants which grow on cellulosic substrates are an example since they result in overproduction of the cellulolytic emzyme complex. Cellulolysis (enzymatic saccharification of cellulose) is favoured, its principle advantage being high conversion efficiency without the production of undesirable side products. However, the cost of microbial cellulase production is high. Reduction of this cost could be achieved through developing regulatory cellulolytic mutants for the commercial production of cellulase.

Cellulase is a complex of enzymes that act together synergistically in the hydrolysis of cellulose. The currently favoured hypothesis of the mode of action is that the substrate is initially hydrolysed by endoglucanases to yield oligomeric intermediates. These are immediately acted on by exosplitting glucanases (glucohydrolase, cellobiohydrolase) which produce glucose and cellobiose. Both tyes of glucanase continue to hydrolyse the residual oligomers and finally cellobiose cleaves both short chain oligomers and cellobiose to yield glucose. Micro-organisms which have the ability to produce high conversion of cellulose possess enzyme preparations with both exo- and endo-splitting components. Their simultaneous occurrence appears to be relatively restricted. Good yields have been obtained from fungal genera including Trichoderma, Fusarium, Sporotrichum and Penicillium.

Detailed analysis of the individual enzyme components from Trichoderma

(T. reesei, T. viride, T. koningi) have been made. The mechanisms controlling the synthesis and action of the total cellulase complex are intricate. Each enzyme is inducible and is sensitive both to catabolite repression and to end-product inhibition. Thus, several approaches to realizing high yielding strains may be used. They include selection of various types of mutants.

As the enzymes of the cellulase complex are repressed by several catabolites (e.g. glucose, glycerol) cellulase catabolite repression resistant mutants should be detectable when grown on cellulosic substrates in the presence of the catabolites. The loss of this control mechanism may lead to hypercellulolytic activity.

Antimetabolite resistant mutants grow on cellulase media in the presence of an antimetabolite of glucose (2-deoxyglucose). They hydrolyse cellulose to yield glucose and thus circumvent the use of 2-desoxyglucose. In contrast, wild strains can only utilize the toxic 2-deoxyglucose and are killed. Resistance to this antimetabolite can arise through several mechanisms including that of catabolite repression resistance (in certain instances) or inability to take up 2-deoxyglucose i.e:

The enzyme activity of end-product resistant mutants is not inhibited in the presence of high concentrations of glucose or cellobiose which is a useful characteristic for obtaining a high degree of cellulose saccharification.

Hyperenzyme production may be achieved as well through derangement of control of enzyme synthesis at cellulase positive revertans from cellulase negative mutants as through derangement of control of secretion at mutants obtained through resistance to membrane active antibiotics (secretory mutants). It should be emphasized that high yielding cellulase strains can be selected also among constitutive mutants (non-induced colonies in a cellulose agar).

It is possible to develop effective strains not only by screening procedures but also by genetic manipulations. They include fusion of microbial protoplasts which may offer for industrial microbiologists one of the new approaches to effect gene transfer. Protoplast fusion may prove to be an effective technique for increasing the frequency of intraspecies hybridization in organisms in which natural mating is a rare occurrence as it is in many species of the Actinomycetes and Streptomyces or for instance in some yeast strains because of their polyploid nature and absence of mating type characteristic (Brewer's yeast strains). The natural barriers to hybridization between dissimilar organisms can often be broken down by the preparations of protoplasts - bacterial or fungal cells whose tough outer walls have been removed to expose the thin cell membrane. Because cell membranes have about the same composition in most species, protoplasts of differenct species can be induced to use and form a hybrid cell, exposing their genes to recombination. The first step in protoplast fusion is the removal of the cell wall with lytic enzymes such as extracts of snail gut or enzymes from various micro-organisms in case the of yeasts or the enzyme lysozyme for the digestion of bacterial cell walls. The protoplasts are osmotically fragile and only remain intact if maintained in a medium of high osmotic pressure (sorbitol, manitol, KCl, etc.). After thorough washing to remove all traces of the protoplasting enzyme, the protoplasts are suspended in the fusing agent which consists of polyethylene glycol (PEG) and calcium ions in buffer and then mixed with protoplasts from a strain with different genetic characteristics. After fusion the fused protoplast must be induced to regenerate its cell wall and begin cell division. In the resulting hybrid cell the DNA of the parents may be recombined. The fusion is not specific enough for transfer of a special gene unless the method is combined with further selection steps.

The most promising new approach to effect gene transfer is recombinant DNA-technology (R-DNA technology) resulting in a direct effort to create the desired guided genetic change. It offers a means of overcoming the non-specificity of fusion. It is another non-sexual technique for achieving genetic recomination. It also enables one to design microbial strains with genetic capabilities to synthesize end-products which otherwise could not be generàted by microbial fermentation.

Recombinant DNA is a hybrid molecule formed by joining two different DNA molecules. In practice pieces of DNA are split from parent molecules at specific nucleotide sequences by means of restriction endonucleases, inserted into a proper vector (plasmid or phage DNA) and introduced into a host microbial cell where the foreign DNA sequence is replicated (cloned) and eventually expressed. The basic requirements for the in vitro transfer and expression of foreign DNA in a host cell can be summarized as follows:

- a vector DNA molecule small, easily prepared, capable of entering and replication within a host cell
- a method of splicing foreign DNA into the vector
- a method of introducing the hybrid DNA recombinants into the host cell and a procedure for selecting the presence of the foreign DNA
- a method of assaying for the foreign gene product.

R-DNA technology has been one of the most rapidly developing areas of science since its first demonstration in 1973 (Cohen and co-workers). Great advances have appeared over the past five years from the first demonstration of the chemical synthesis, cloning and functional expression of the human hormone somatostatin (Itakura and co-workers, 1977), to recent cloning and functional expression of synthetic human insulin in Escherichia coli (Goeddel et al., 1979) and the synthesis of human interferon related peptides (Gilbert and Villa-Komaroff, 1980).

R-DNA technology is also undergoing rapid development in streptomycetes (Bibb, Schottel, Cohen, 1980) which produce 60% of the known antibiotics and in yeast strains improvement programs (Hinnen et al., 1978; Beggs, 1978; Broach et al., 1979).

The applications of new genetic technologies appear numerous and undoubtedly will receive considerable attention over the the next years offering exciting prospects for the systematic construction of improved strains.

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MICROBIAL PROTEIN PRODUCTION ON PLANT WASTES OF INDUSTRY AND AGRICULTURE

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The forecasts of authorities about protein provision in the future by traditional methods of production are not optmistic. The modern state of the microbiological industry suggests that an important role in the solution of this problem may be by microbial synthesis with the utilization of cheap native organic compounds and wastes of industrial and agricultural production as raw materials.

Cellulosic- and starch- containing wastes of industry and agriculture because of their widespread occurance, accessibility and cheapness can be the main raw materials for feed and food protein production by means of microbial synthesis. Our investigations were directed to study the raw materials and to develop technological processes of bioconversion of cellulosic and starch-containing raw materials into protein.

The variety of cellulosic-containing raw materials demands different approachs to the choice of bioconversion methods. Depending upon raw material properties, its location and economic factors, etc., cellulosic- and starch-containing raw materials intended for bioconversion processes have been divided into two groups. Raw materials of the first group are intended for industrial biological processing. Depending upon physical properties and chemical composition individual types of raw materials have been recommended by us for solid phase or submerged culture methods of production.

Raw materials of the second group are recommended for direct processing at collective farms, namely at fodder supply centres.

In our work we have used those wastes of industry and agriculture that are of great interest for remote regions of the country. At the present time these sorts of raw materials although used as fodder addition are of little efficiency because of their low protein content. Corn brans and feed grains are exceptions. However we have considered that it is expedient to investigate bioconversion of not only cellulose but also starch, taking into consideration that there is some abundance of starch products in relation to protein supplies.

Thus we have used cotton stalks, feed grains, corn brans, straw, untreated vegetables, beetroot pulp, remains from scutched* fibrous crops, fruit- and berry-pulp (Table 1).

* scutch: process of separating the woody fiber from (flax or hemp) by beating.

Table 1. Protein formation by microbial culture on cellulosic- and starch-containing substrates.

	substrate used	producer	Culture before process	Protein content, % in finished product	
1.	cotton husks	microscopic surface fungi		3- 4	18-20
2.	feed grains	microscopic surface fungi		12-15	35-38
3.	corn brans	microscopic surface fungi		15-17	35-40
4.	vegetables unconditioned	bacterium	submerged	3- 5	20-25
5.	beetroot pulp	microscopic fungi, yeasts	submerged	5- 7	35-45
6.	fruit-and-berry pulp	yeasts	submerged	5- 7	35-40
7.	straw	yeasts	submerged	2- 3	10-12
8.	remains of scutched fibrous crops	microscopic fungi, yeasts	submerged	2- 3	20-25

Such raw materials as cotton husks, brans and crushed feed grain are suitable raw materials for the process of solid phase culture by their structure. By utilizing microscopic fungi *(Fusarium, Sporotrichum, etc.)* higher protein contents have been achieved in comparison with the initial substrates by 2,5 - 4 times.

Beetroot and fruit-and berry pulps fresh-squeezed contains 95% of moisture. It seems expedient to carry out the bioconversion process of some raw materials by means of submerged culture because drying and granulation of the substrates used would require an additional power supply.

Utilizing both pure culture and mixed culture as producers we have increased significantly the protein content in the finished product by comparison with the initial substrate.

Taking into account that it is not expedient to transport such agricultural wastes as straw, a process of biological processing of straw that is simple in operation and easy in realization has been developed at our research institute. This process may be realized at any feed centre using s' ..dard equipment.

The process involves preliminary fermentative hydrolysis of straw followed by the growth of yeast on the hydrolyzates. The technological process of bioconversion of flax scutch (wastes of the flax processing industry) has been developed on the basis of the method mentioned above.

Economic investigations have shown solid phase culture of producers are the most progressive method for bioconversion of cellulosic- and starch-containing raw materials into protein. However realization of the large-scale protein production on the basis of microbial growth on solid substrates is connected with certain difficulties.At the present time highly productive

mechanized plants are not available.

We have carried out investigations of solid phase culture in a mechanized pilot plant that has been developed at the research institute "VNIIbiotechnique." The growth of microbial cultures at this plant are carried out in a medium layer of 300-500mm.

The plant consists of a vertical cylinder divided into sections by perforated plates fixed with cantilevers on rotary axes. Stirring arrangements are located inside the section and provide slow stirring to ensure a uniform of layer height and air distribution which does not allow the formation of dead zones. At the top the plant is aseptically connected to a sterilizer. Air for the aeration inlet to each section under the perforated plate is provided at given temperature, moisture and volume. Dissapation of heat liberated during the growth of the culture is achieved by means of a cooling agent fed into the plant's jacket. Transfer of the culture medium from the upper section to the lower one is performed automatically at a given time interval. At this moment the plate turns at 90^0 and then returns to the initial location. The culture is received in the conical part of the plant's base and is charged into the accepting bunker of the dryer.

Such a plant is able to carry out a semi-continuous process by means of the solid phase fermentative method. The culture grows in a closed atmosphere and is aerated through the performated plates by convective air flows in the medium due to pressure differential and the porous structure of the medium.

In this case gas does not flow along the surface of the medium layer but is distributed through the whole volume; the regime of volume aeration is obtained and this regime is able to ensure convective and diffusional mass heat exchange through the whole height of the medium.

Application of this method of aeration of the cultural medium enables the height of the medium layer to be increased up to 300-500mm.

A special feature of microbial culture using the solid phase method is the generation of a significant quantity of heat. The methods of indirect calorimetry of heat production by microbial culture is based on microbial respiration data and are rather approximated. Heat production of a microbial population is an integral measure of the calorific effect of oxidative-reduction processes in the biological system as a whole.

Data from investigations of thermoproducton by micro-organisms by direct and indirect calorimetry has been established by a number of authors.

For direct measurement of heat generated during microbial growth a special microbial calorimeter has been developed, constructed and fabricated at the research institute "VNIIbiotechnique." Specific features of microbial culture under industrial conditions are taken into consideration by this apparatus. The operating vessel has been made in a form of thinwalled duralumuin glass with a changeable thermo-isolated porous top. Dissipation of excessive amounts of physiological heat from the vessel to the heat exchanger has been produced through a thermomer. The sensitivity of the thermomer was $1.13 m^2V/Wt$. Investigations were carried out under isothermal conditions and before each test the heat balance was set in the calorimetric system. The constance of the zero point of a signal from the thermomer was evidence of this. Such a system led to the recording of thermal flow in exo- and endothermal and combined reactions with high accuracy.

The design of the calorimetric apparatus permitted chemical and thermal sterilization of operating vessels.

From the thermograms of solid phase culture of *Sp.pulverumentum* on cotton husks, the following may be noticed. Use of the direct calorimetric

method enables information to be obtained on the culture growth under surface conditions as these thermograms are in good correlation to the protein formation and the rate of substrate consumption.

The magnitudes of specific thermo-production of *Sp.pulverumentum* when growing at different temperatures from 30^0 to 43^0C have shown that the maximal heat loss is observed at 40^0C and this correlates with maximal protein formation, reducing sugars and substrate consumption (Table 2). Certain regularities of heat loss occur during culture growth on given substrates. At the present time we have not sufficient data to draw the whole picture of the relationship between complex processes of the micro-organism's metabolism and thermal energy obtained by these processes. Experimental data shows that this relationship is not random and using the thermograms it is possible to control and optimize the process.

Table 2. Influence of the microscopic fungi *Sp.pulverumentum* culture on the protein formation, reducing sugars and the rate of substrate consumption (substrate - cotton husks).

	features studied / temperature of the process T°C	30	33	37	40
1.	Protein content, %	9.7	7.6	12.6	16.5
2.	Reducing sugar content, %	12.0	9.2	10.3	13.9
3.	Substrate dry weight loss, %	11.0	12.0	12.0	19.0

The feed product obtained on cotton husks contained crude protein up to 20% cellulose less than 20% and lignin - 26%. The degree of cellulose bioconversion has been 45-48% and lignin - up to 30% (Table 3).

Table 3. Features of the initial substrate and fodder product obtained by surface producer culture on cotton husks.

	Component	content, % before the process	in finished product
1.	crude protein (N x6, 25)	3.66	19.5
2.	cellulose	31.45	20.0
3.	lignin	30.65	26.2
4.	polysaccharides easily hydrolyzed	24.95	16.5

In the product 80% of the nitrogen content was protein. The new fodder product contains vitamins, enzymes and lipids (Table 4). The presence of cellulolytic enzymes in the product obtained is a positive result as in the utilization of such feed together with roughage it may be expected that introduction of cellulase will increase the digestibility of the product obtained.

Table 4. Features of the fodder product obtained by surface producer culture on cotton husks.

nitrogen compounds, %	
total nitrogen	3.1- 3.4
crude protein (N x6.25)	19.5-21.0
protein nitrogen	2.5-2.75
ammonia nitrogen	0.5-0.7
vitamins, mg/kg	
riboflavin	6.25-7.0
thiamin	2.5-3.0
nicotinic acid	34-35
biotin	0.5-0.7
enzymes, unit/g	
C_1	20
C_x	125
lipids (total), %	2-3.5

The amino acid content of the fodder product obtained on cotton husks is near to the FAO standard (Table 5). Toxicological investigations show the product is harmless. Thus the protein product obtained may be recommended for feed by its quality.

Straw is one of the most widespread agricultural wastes. Untreated straw has low nutritious and biological value. The conversion of straw would therefore be worthwhile. The biological method developed at the research institute "VNII-biotechnique" led to an increase in the protein content of straw from 2-3% to 10-12%. If the digestibility of the protein from native wheat straw is 20% its digestibility is 70% after biological processing.

Utilizing straw fermentative hydrolysis at 55^{0}C and atmospheric pressure it is possible to operate the process with standard equipment. The tests of feeding cattle with straw biologically processed show that utilization of this fodder product increases daily milk-yield, fat content of the milk and decreases the concentrated fodder consumption for each litre of milk obtained.

At the present time this method has been introduced in different regions of the country and high economic efficiency has been obtained according to the data received from some collective farms.

Table 5. Amino-acid content of fodder protein product obtained by the surface producer culture on cotton husks.

	amino acid	amino acid content, % to protein		
		protein product on cotton husks	FAO standard	wheat
1.	lysine	7.05	4.2	2.7
2.	histidine	2.78	-	-
3.	cystine	13.72	2.0	4.3
4.	arginine	4.3	-	-
5.	aspartic acid	13.35	-	-
6.	threonine	11.85	2.8	3.3
7.	glutamic acid	11.94	-	-
8.	serine	11.26	-	-
9.	proline	5.34	-	-
10.	glycine	7.50	-	-
11.	alanine	8.95	-	-
12.	valine	6.35	4.2	3.5
13.	methionine	2.50	2.2	4.3
14.	isoleucine	4.38	4.2	3.0
15.	leucine	4.60	4.3	4.0
16.	tyrosine	2.16	2.8	-
17.	phenylalanine	2.32	2.8	9.3
18.	tryptophan	1.20	1.4	1.0

THE PROTEIN PROBLEM AND HIGHER FUNGI MYCELIUM

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1. Introduction

The traditional sources of protein such as meat, milk, eggs, fish and plant proteins are not able to solve the protein problem on our planet, because of the constant increase of the world's population. Besides the insufficiency of proteins concerning quantity, an effect on man's nutrition is caused by the inadequate amino-acid composition of some proteins mainly those of plant origin.

Another source of proteins is that of microbial origin. These microbial proteins can be from bacteria, yeast, moulds, or the mycelium of higher fungi. Microbial protein is not new to scientists but it has been looked upon upto now mainly as a protein for animals feeding. However, the protein feed to animals is only converted to animal protein for man's food with an efficiency of 20-25% the remaining 75% is used as an energy source or is turned into non-nutrient protein such as skin, fur, horn, inner inedible parts, etc. Therefore for solving the protein problem on a world scale, we should think of a more rational method for using the microbial protein as man's food than using it only for animal feed. When speaking of microbial protein as man's food at this stage we should consider mainly the mycelium of higher fungi for at least for two reasons:

1. Mushrooms since times immemorial have been used as food and man has adapted himself to this kind of protein.
2. For phsychological reasons.

The requirements of fungal mycelium have some things in common with the rest of the lower organisms although there are also some special aspects. The basic nutrient media for fungal mycelium is molasses with addition of nitrogen and phosphorus sources but mycelium can be successfully grown on other carbohydrate sources such as glucose, spirits, whey, etc. Parameters of the development of fungal mycelium do not differ basically from the rest of the lower organisms. The development cycle depends on the quantity of growing material and varies from 7 to 48 hours. It can be developed by interrupted, semi-uninterrupted or uninterrupted methods. Fungal mycelium is easily filtered by all kinds of facilities. It is washed in drinking water and is obtained in two forms - moulded and dry.

We have obtained mycelium in two forms - moulded and dry because of the characteristics of its usage. The moulded one is in blocks of 10 kgs containing dry substance of 26-28%. It has a white colour with a slightly grayish shade and

fibrous structure with a taste of yeast or more exactly one would say without any specific flavour. Dry fungal mycelium contains 93-94% dry substance, powderlike or slightly fibrous structure depending on the method of drying and grinding; of white to gray colour with a slightly cream-coloured shade and a mushroom taste or the taste of baked flour.

Our developments have been concerned with industrial applications. The biochemical aspects fungal mycelium of the strain PS-64 are given in Table 1.

Table 1. General chemical composition of mycelium

Raw protein	54-58%
Pure protein	44-46%
Carbohydrates	22-24%
Lipids	4%
Mineral composition	7%
Nucleic acids	6-8%
Vitamin B_1	9 mg %
Vitamin B_2	39 mg %
Vitamin B_{12}	0.140 mg %
Digestibility of protein	83%

Medico-biological investigations on fungal mycelium were done in the course of many years for acute toxicity, carcinogenity, terratogenity, pathomorphological investigations, allergy, etc. The experimental results showed that no changes or reaction are observed in the test animals.

As a result of the many years of medico-biological investigations at the Higher Institute of Food in the People's Republic of Bulgaria and the Soviet Union, a medical conclusion was given that fungal mycelium can be used for man's food.

Fungal mycelium as we have seen from the biochemical composition is a rich source of protein, with a well balanced amino-acid composition and high digestability. The question is raised, under what kind of form the fungal mycelium can be used as a nutrient product, in what ways, in what percentage, its palatability and appearance.

Numerous experiments were done to investigate these aspects and it was found that fungal mycelium can be used in the mould or dry state in different ways, the most important of which are the following:

1. Meat industry - for producing different kinds of sausages and other meat products.
2. Milk industry - for producing different kinds of melted and smoked cheeses.
3. Producing different kinds of canned vegetables enriched with protein.
4. An alternative method of using the fungal mycelium for solving the protein problem is by "self-enriching" of fodder flours with protein through solid-phase fermentation.

A technology has been successfully developed for improving fodder flours using fungal mycelium. For this purpose it is necessary to saturate the fodder flours with nitrogen sources. The prepared nutrient media is then inoculated with a liquid substratum of fungal mycelium and is placed in a special apparatus for cultivation. The cultivation lasts about 36 hours.The fodder flours are enriched by the mycelium with 5-7% pure protein.

Depending on the basic contents of protein in the substratum e.g., maize flour with about 10 % pure protein, a fodder mixture of 15-17% pure protein is obtained. Such fodder mixture contains more pure protein than the normal for feeding pigs and cattle and is adequate for the normal for feeding of poultry.

The experiments with white rats showed that such fodder mixture gives a better growth than the balanced fodder mixtures with soya flour, fodder yeast, or other protein sources.

So fungal mycelium can help to solve the protein problem both in our country and on a world scale in two directions - through its direct use as human food and through using it to obtain protein enriched fodder flours.

Table 2. Comparative data according to the amino-acid composition of fungal mycelium with other protein sources

	mycelium PS-64	beef meat	casein	soya flour	standard protein FAO
Lysine	8.5	8.4	8.4	6.4	5.5
Threonine	5.3	4.0	5.0	3.8	4.0
Valine	6.0	5.7	7.4	5.0	5.0
Isoleucine	5.1	5.1	6.2	6.4	4.0
Leucine	7.2	8.4	9.4	6.6	7.0
Tryptophan	1.4	1.1	1.2	1.2	1.0
Methionine	1.9	2.3	2.0	0.7	3.5
Cysteine	0.9	1.4	0.3		-
Phenylalanine	3.9	4.0	5.1	4.8	-
Tyrosine	3.4	4.0	6.4	3.1	6.0
Total	44.1	44.4	51.4	38.0	36.0
Histidine	2.9	2.9	3.2	2.3	
Arginine	5.8	6.6	4.2	6.0	
Asparaginic acid	10.3	8.8	3.7		
Serine	4.8	3.8	6.4		
Glutamic acid	16.2	14.4	22.9		
Proline	4.0	5.4	10.9		
Glycine	4.8	7.1	2.0		
Alanine	7.6	6.4	3.3		

THE PROTEIN PROBLEM IN THE COMPLEX FRAMEWORK OF BIOMASS UTILIZATION

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1. Introduction

Grain crop and meat production play a decisive role in Hungarian agriculture and in the foreign trade of agricultural products. Based on the huge quantity of grain crop produced in the country, a high level of output has been reached in animal husbandry providing the possibility to export live animals, meat and processed animal products. In order to keep this system functioning, however, large quantities of protein feed has to be imported for hard currency. Due to the relatively expensive technologies applied in animal husbandry, the economics and competitiveness of meat production and exports are threatened by the high prices of imported protein feed and by relatively depressed export prices of meat. Recent trends in exports and imports of these products are shown in Table 1.

Table 1. Feed imports and meat exports (unit: 1000 tons)

Imports	1960	1970	1975	1980
Feed of animal origin	2.4	85.2	65.8	60.1
Feed concentrate	3.2	20.3	19.2	4.8
Feeds coming from vegetable oil industry	31.5	336.4	505.3	620.3
Fodder cereals	53.3	141.4	172.6	100.2
Exports	**1960**	**1970**	**1975**	**1980**
Flesh	23.8	43.4	107.6	160.9
Slaughtered poultry	15.4	56.6	103.9	135.2
Sausage products, salami	3.2	7.6	8.2	10.8
Beef cattle	68.2	112.9	105.0	64.6
Pig	12.7	2.7	19.7	56.1
Sheep	4.0	23.9	21.7	27.0

In connection with the problems mentioned above, we have to take into consideration that:

- enormous quantities of by-products and waste materials - containing, among others, a great amount of valuable protein - are lost in plant production, animal husbandry and the food industry every year;
- the proportion of animal protein consumption in Hungary is far above the level justified by physiological need.

In order to illustrate these problems, a "traditional" and a "non-traditional" pattern of production and utilization are outlined in Fig. 1 and 2.

These figures show that there is a need for a twofold change in our attitude:

(i) Social aspects: In Fig. 2 we have a different structure and new paths in the product flow. It is well-known that one unit of protein of animal origin for human consumption requires approximately seven times more energy than protein of vegetable origin. The question arises: How the diet structure can be changed in order to increase the proportion of vegetable and non-conventional protein sources. This is going to be a difficult problem, because it usually takes a long time to change habits and traditions. However, if the lower energy input will be reflected in the prices of non-traditional protein food then this might facilitate these changes.

(ii) Technological aspects: in order to substitute, at least a part of the imported protein feed by waste materials and by-products of plant production, animal husbandry and food industry, it is an urgent task to elaborate new technologies for processing these materials into animal feed. Here again, the problem is, that a great amount of fixed assets have been invested in the existing technologies and if we reduce the proportion of protein feed on the one hand and increase the share of by-products and waste materials on the other, then this will result in a slower rate of augmentation in weight, which, in turn, leads to a lower return on investments. Therefore, in this respect, non-traditional feed production technologies should be investigated in the complex framework of feeding technologies. (One solution to this problem might be organic agriculture, but we have very little information on this alternative at present.) Returning to our Fig. 2. for a moment, it is obvious, that there exists many other protein sources and alternatives to utilization. For example: since the by-products are waste materials of biological origin and have a considerable energy content, they can be utilized as energy resources as well. Therefore, it is necessary to investigate the protein problem in the overall framework of biomass production, transformation and utilization.

2. Short Summary of the Biomass Project

The Hungarian Academy of Sciences, cooperating with the Ministry of Food and Agriculture, the State Office for Technical Development, the Ministry of Industry and various other relevant bodies, is preparing a national survey on the complex utilization of materials of biological origin (biomass).

Work was begun in May 1981 and is expected to be completed by spring 1983. At present 20 working groups consisting of several hundred researchers and field officers, are studying new ways of utilising the biomass, in the food industry, for feeding purposes, in the timber industry, in the chemical industry and in many other spheres, including non-conventional protein production and utilization technologies.

In recent years growing attention has been paid all over the world to the need to take stock of the available natural resources and to develop new ways of utilising them.

The scope of these surveys is no longer restricted to energy resources and raw materials, but has been broadened to include renewable resources as well.

The renewable character of the biomass gives it a special place among the natural resources, since its potential can be increased by rational utilisation. However, this necessitates a knowledge of the reserves latent in the biomass and of the possibilities and limits involved in their utilisation. Nor must it be forgotten that, in addition to the role played by biological resources in food production and in maintaining the environmental balance, the biomass is becoming more and more important as a source of raw materials and energy.

The basis for all this is formed by plant production and forestry.

The growth rate achieved in plant production in Hungary over the last two decades was the second fastest in the world. This rapid development was primarily due to the intensification of agriculture. Less appreciation was given to the natural resources available to agriculture, since they were overshadowed by the technical development.

There are two possible paths for future development:

- the further intensification of agriculture, requiring an increasing supply of materials, implements and energy, the majority of which must be imported. The question arises: *Is it worth applying this method, can the natural conditions ensure the biological background required for further development*?
- the rational utilisation of the latent possibilities to be found in the natural environment.

If this latter course is to be followed, it is essential to determine the effect of ecological conditions on plant production, the extent and limitations of the agroecological potential and ways of overcoming these limitations.

Obviously, a knowledge of the agroecological potential is also needed for the elaboration of an intensive development strategy.

A survey of Hungary's agroecological potential was carried out in the 1978-1980 period under the supervision of the Hungarian Academy of Sciences. Several hundred researchers and field officers participated in the work.

Details of the survey will be found in Lang (1981) and Harnos (1982). We also use some results of the Hungarian Agricultural Model (HAM), which was elaborated in cooperation with the Food and Agricultural Program at IIASA (see Csaki, 1981).

It is clear from the survey of agroecological potential that plant production could be significantly increased.

The other course of development would be to find ways of utilising by-products and waste materials. In addition to the main product, a significant quantity of by-products and waste materials, with a mass equivalent to two-thirds of the main products, is also produced.

According to statistics compiled by the Hungarian Central Statistical Office, approximatley 55 million tons of dry matter of vegetable origin was produced in Hungary in 1980, of which over 20 million tons was written off as by-products or waste material. Naturally this does not mean that a large proportion of this has not been utilised up till now. The point is, that if we increase plant production, there will be a corresponding increase in the mass of by-products and waste materials awaiting utilisation.

Let us compare the development potential determined on an ecological basis with the expected trend in domestic comsumption.

At present 70-75% of the agricultural production is needed to satisfy domestic demands, while the rest is exported.

Since the population of Hungary will not increase by the turn of the century, there will only be structural changes in the consumption. Consequently, with the developmental potential assumed in the prognosis, half the agricultural production will be sufficient to cover domestic demands in the year 2000.

The data presented apply first and foremost to the primary biomass production. If the transformation and utilisation of this biomass is also considered, a certain lack of proportion becomes visible, such as Hungary's protein deficit, but also new reserves, such as animal carcases, food industry waste materials, farmyard manure, etc., the utilisation of which would have a favourable influence on the agricultural export-import situation, even if the present plant production and animal husbandry structures were maintained.

The biomass program aims to clarify the internal relationships of the biomass production-transformation-utilization system and to determine the future function and role of this system in connection with the rational use of renewable and non-renewable resources.

In investigating the system, questions of the following type were chiefly raised:

- how should biomass production be organised in time and space as a function of changes in the external conditions?
- what non-renewable resources could be substituted if the biomass were fully exploited?
- what economic consequences, leading to a simultaneous improvement in the environment, could be expected if a complete utilization chain were to be constructed (e.g. full utilization of by-products and waste materials, etc)?
- how sensitively will the system respond to changes in the external conditions and how can the detrimental effects of drastic changes be eliminated or moderated?
- which part of the biomass is most suitable for producing food, feed, energy or other raw materials?
- of the wastes and by-products under the presently used technologies, which part has the advantage of economical utilization as a protein source (on the basis of protein content), as energy resource (on the basis of calorific value), as raw material in the chemical industry, as fertilizer, etc.?

In order to answer these questions, when elaborating long- term plans for utilising the biomass, possibilities for the development of the following sectors were considered:

- plant production, including forestry,
- animal husbandry,
- the food industry,
- the processing and utilisation of industrial raw materials, and the by-products and waste materials of plant production, animal husbandry and the food industry,
- the effect which production and processing have on the environment, and if this is negative, how it can be overcome.

It can be seen from this brief outline that the program involves the analysis of a complex system embracing agriculture, the processing industry (including the food industry) and environmental protection, and the elaboration of various

ways of developing them.

3. The Logical Structure of the Model System

This paper is not designed to provide a detailed knowledge or analytical description of the whole problem therefore only the logical structure of the model is presented below. (See Lang and Harnos, 1982, for the details.)

The description of the problem was divided into two parts: a *system of conditions* and a *production system*. This is illustrated schematically in Fig. 3.

The system of conditions gives a formulation of the economic conditions and goals, while the production system gives a description of the production, processing and utilisation of the biomass.

The production system is described by means of a hierarchical family of models (Fig. 3), constructed in such a way that the operation of the various levels of biomass production and utilisation can be analysed independently of each other; it is also possible to link only certain sectors and thus analyze the effects exerted by their production structures on each other (e.g. plant production, animal husbandry, etc.).

The hierarchy is manifested in two ways.

(i) The complete process of biomass production and utilization is described in aggregated form in a control problem. The alternative paths of development formulated in the system of conditions form the limits of the model.

Calculations made using this model specify the conditions for the indidvdual sectors for the whole period under investigation. In the second level the sectoral models are studied in detail.

(ii) A certain hierarchy is also necessary within the sectoral models. Due to their detailed nature, models describing the individual sectors cannot be linked up and solved as a single system. The equilibrium between the sectors is ensured by the aggregated model, but the limits this implies provide a fairly large degree of freedom for the sectoral models. The detailed calculations lead to results deviating from the stipulated paths of development. These main links in the system are presented in Fig. 4.

4. Animal Husbandry and the Protein Problem

In the system describing problems outlined in the previous section, special emphasis is given to the flow of materials which have substantial protein content and/or can be used for energetic purposes. The importance of these materials is shown in Table 2.

The total amount of these by-products, which are suitable for feeding purposes, is equivalent to:

- bioenergetic content of maize (grainstalk) grown on 406,000 hectares (5.0 tons/hectare yield);
- protein content of maize (grain only) grown on 336,400 hectares;
- the feed of 400,000 tons of beef cattle kept in stalls
- 2.6 million tons of oil equivalent on the basis of its combustion heat (excluding sugarbeet-top and green pea stalk);
- calorific value of 485,000 tons of oil, when it is used for biogas generation (plus 70% of its organic matter content can be used as fertilizer);

Table 2. By-products suitable for feeding purposes.

By-Product	Potential Yield	Energy demand of gathering in	Bioenergy Content	Protein Content
Feeding straw	836.6	124.7	2741.5	6.7
Pea-straw	145.5	22.7	549.3	4.1
Corn-stalk	10,265.9	630.6	29,846.4	123.2
Sugarbeet top	419.2	55.9	1,066.9	5.0
Green pea stalk	555.6	80.0	814.3	5.4
Total	-	913.9	45,078.4	144.4

- 863,000 tons oil equivalent of methanol, when it is used to produce industrial alcohol.

Similar calculations have been made for other by-products of plant production, which are not suitable for feeding purposes, and for the by-products and wastes of animal husbandry (manure, animal carcases). Waste materials produced in different sectors of food industry are examined in the same way.

In this section the animal husbandry sector and its connections to other sectors are discussed, because this sector is the most important one from the point of view of protein production (both conventional and non-conventional technologies) and utilization.

Similarly to the other sectoral models, animal husbandry is modelled as a control problem. As it is usual, conditions for the operation of the system and the internal relationships of the sector are described by state variables, while the activities in the system are regulated by control variables.

The state variables in the animal husbandry model are as follows:

- variables describing the conditions of plant production
- system of conditions for animal husbandry (breeding stock, buildings, machinery, etc.)
- feed producing plants.

Control variables describing the control of the system are as follows:

- investment goods (resources);
- import openings.

If the scope of investigation is narrowed down to animal husbandry and elements in direct connection with it then the constraints for plant production and output from animal husbandry can be handled as controls.

Details of the plant production model will not be presented here, but the structure of the model is shown in Fig. 5.

The structure of the animal husbandry is described by

- varieties, and within these:
  - = beef cattle

= cow (milk)
= pig
= poultry
= egg
= sheep

- the level of intensification (small-scale animal husbandry is treated as extensive technology).

Within a given variety, the most important differences are described by the following parameters:

- intensive technologies require a higher level of investments, at the same time
- degrees of feed utilization are higher in these technologies, that is: a smaller amount of protein and starch is required to produce the same amount of meat, milk or eggs. These relationships are shown in Table 3.

Table 3. Specific nutrient requirements.

Product	Specific demand in gr.		Degree of protein concentr.
	starch value	protein	
Required to produce 1kg of milk			
2000 litres	820	110	13.4%
3000 litres	637	92	14.4%
4000 litres	545	83	15.2%
5000 litres	500	77	15.0%
6000 litres	463	73	15.8%
Required to produce 1 kg of eggs			
100 pieces	3,100	617	19.9%
150 pieces	2,290	500	21.8%
200 pieces	1,900	433	22.8%
250 pieces	1,640	397	24.2%
Required to produce 1 kg of pork (live weight, 90 kg)			
8 months fattening period	2,880	420	14.6%
7 months fattening period	2,700	405	15.0%
6 months fattening period	2,500	390	15.6%
5 months fattening period	2,250	375	16.7%
Required to produce 1 kg of broiler			
70 days cramming period	1,800	400	22.2%
60 days cramming period	1,650	380	23.0%
50 days cramming period	1,500	360	24.0%
40 days cramming period	1,350	340	25.1%

The quantity and composition of the feed utilization can be controlled by changing the proportion of varieties in the meat production sector. Changes in the proportion of varieties and the level of intensification, however, can only be induced by different investment activities. This fact is directly manifested in the animal husbandry subsystem.

Investments in the activities providing the feed supply are built in other sectoral models, which are indirectly connected to the animal husbandry model. These activities are, for example, processing animal carcases and wastes from food industry for protein feed, processing by-products of dairy industry for feed, etc. Investigation of these activities cannot be separated from the studies concerned with biomass utilization and the protein problem.

The composition of feed is controlled by the following five parameters:

- starch content
- protein content
- degree of protein concentration
- fibre content
- complements (e.g. lysine)

To produce a given quantity of meat, feed must be supplied in proper quantity and composition (as far as the nutrient content is concerned).

This quantity of feed comes from:

- main products of plant production (cereals, rough fodder, soybean, alfalfa, etc.)
- by-products and waste materials of plant production, which are treated this way at present (corn stalk, sugarbeet-top, etc.)
- waste materials of animal husbandry (meat meal, etc.) - processing requires a certain amount of investments.

Utilization chains, that is, the flow of materials considered in the model are shown in Fig. 6. Processing sectors of the food industry are represented in the model in a more detailed way. Utilization alternatives are built into our model for each phase of processing where sufficient quantity of by-products and wastes are produced. Our intention is to give more alternatives to each type of by-products in order to ensure the production of materials which are actually needed and, at the same time, economic considerations (profitability, optimal allocation of investments, etc.) are enforced this way.

The schematic representation of these problems is shown in Fig. 7. The range and level of activities are regulated by control variables (represented by double headed arrows in Fig. 7). This figure, however, represents static relationships, but in the model the dynamics of the system will be examined. Another problem is, that the by-products shown in the figure represent a great quantity, but a relatively low value and they are scattered in reality. It means that profitability of processing can be ensured only by careful allocation of processing capacities. In order to tackle this problem, our country was divided into regions reflecting the ecological conditions to the biomass production on the one hand, and providing the possibility to investigate regional aspects of the biomass utilization on the other.

5. Expected Results of the Survey

After this brief summary some indication should be given of the expected results.

The analysis of biological resources and their utilization as a single system will give a sound basis for comparing them with non-renewable resources and for elaborating a coordinated development strategy for the natural resources of the country.

The period of examination is 20 - 25 years; however, the results will be used not only to elaborate long-term paths of development valid for the whole period,

but also to study, on the basis of the present situation, what reserves can be exploited within the next few years, and in which areas rapid economic results can be achieved through the more rational use of biological resources.

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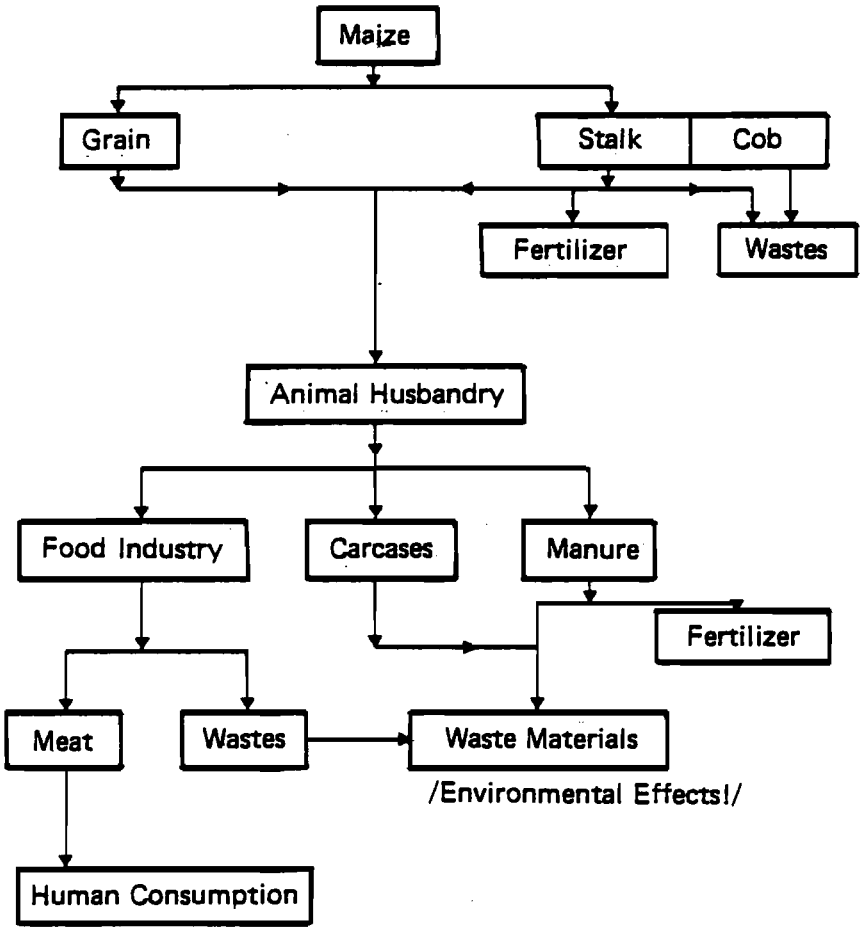


Fig. 1. "Traditional" pattern of production and utilization

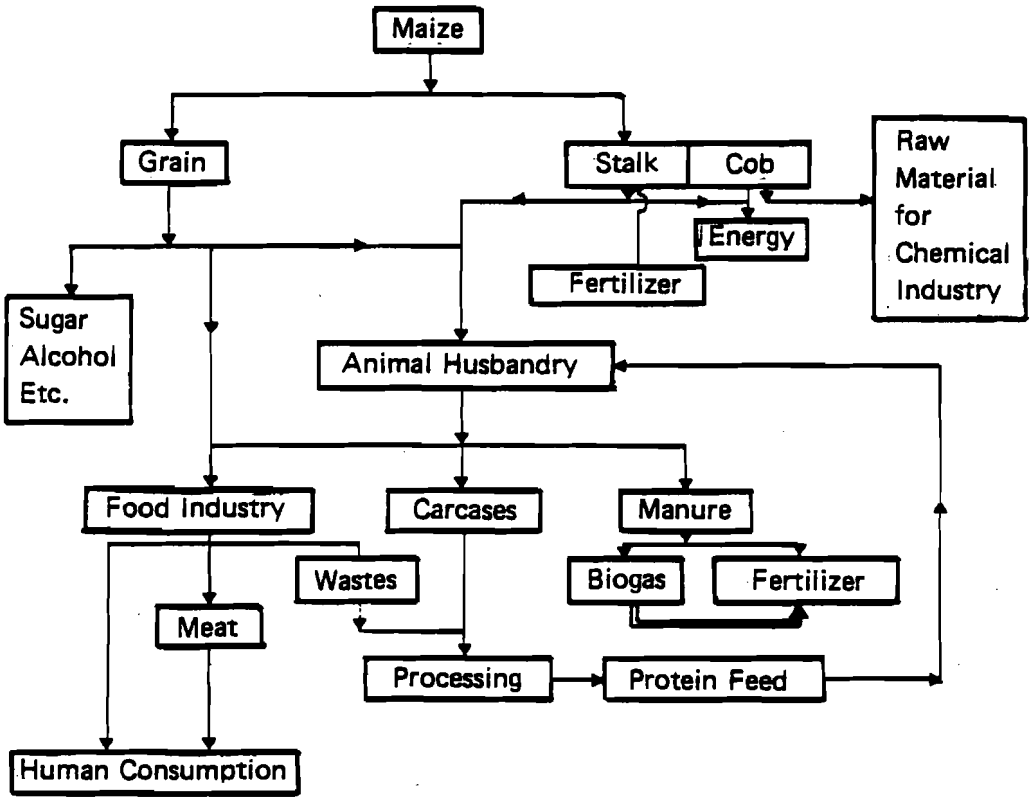


Fig. 2. "Non-Traditional" pattern of production and utilization

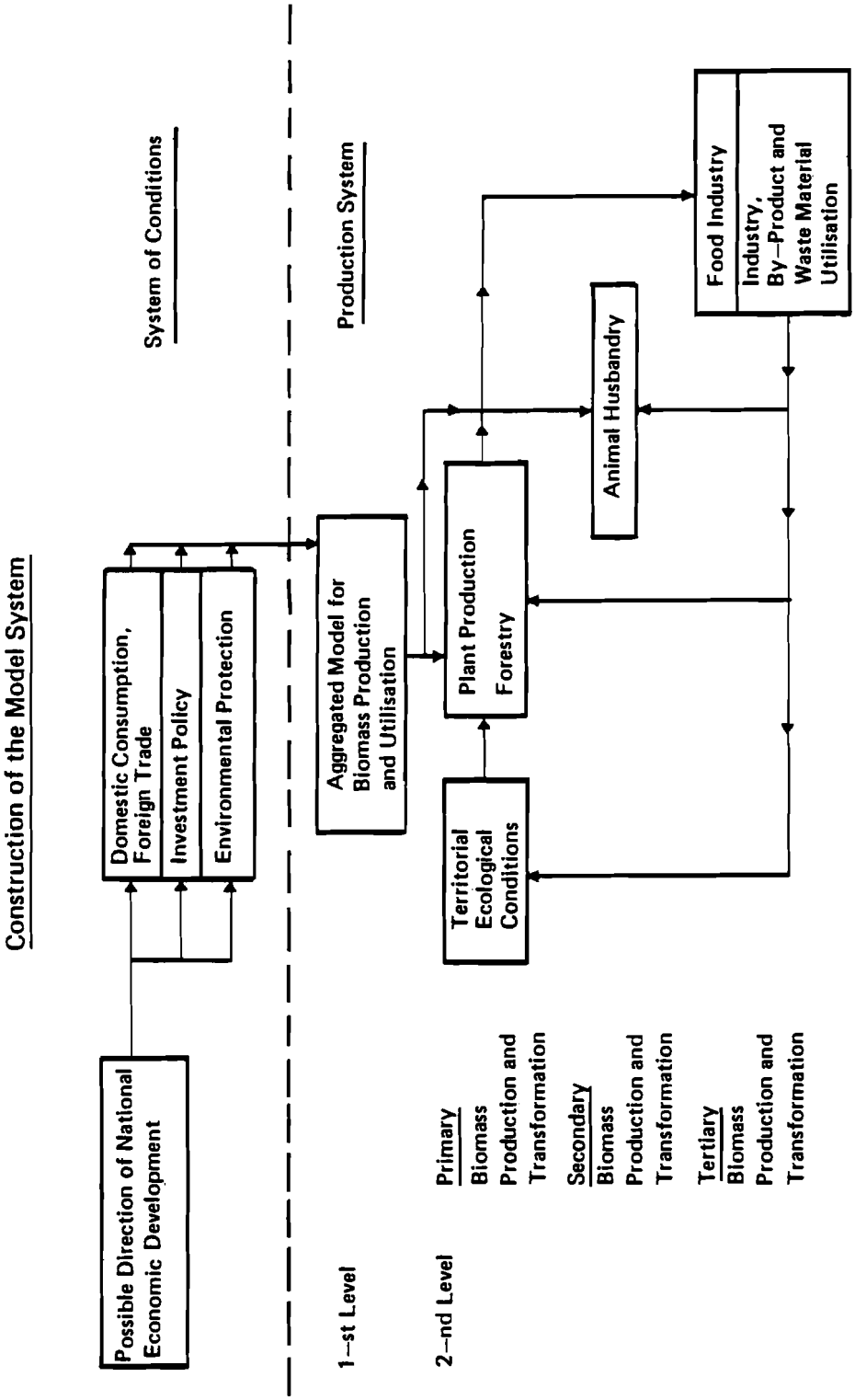

Construction of the Model System
System of Conditions
Production System
Possible Direction of National Economic Development
Domestic Consumption, Foreign Trade
Investment Policy
Environmental Protection
Aggregated Model for Biomass Production and Utilisation
1–st Level
2–nd Level
Territorial Ecological Conditions
Plant Production Forestry
Animal Husbandry
Food Industry
Industry, By–Product and Waste Material Utilisation
Primary Biomass Production and Transformation
Secondary Biomass Production and Transformation
Tertiary Biomass Production and Transformation

Fig. 3

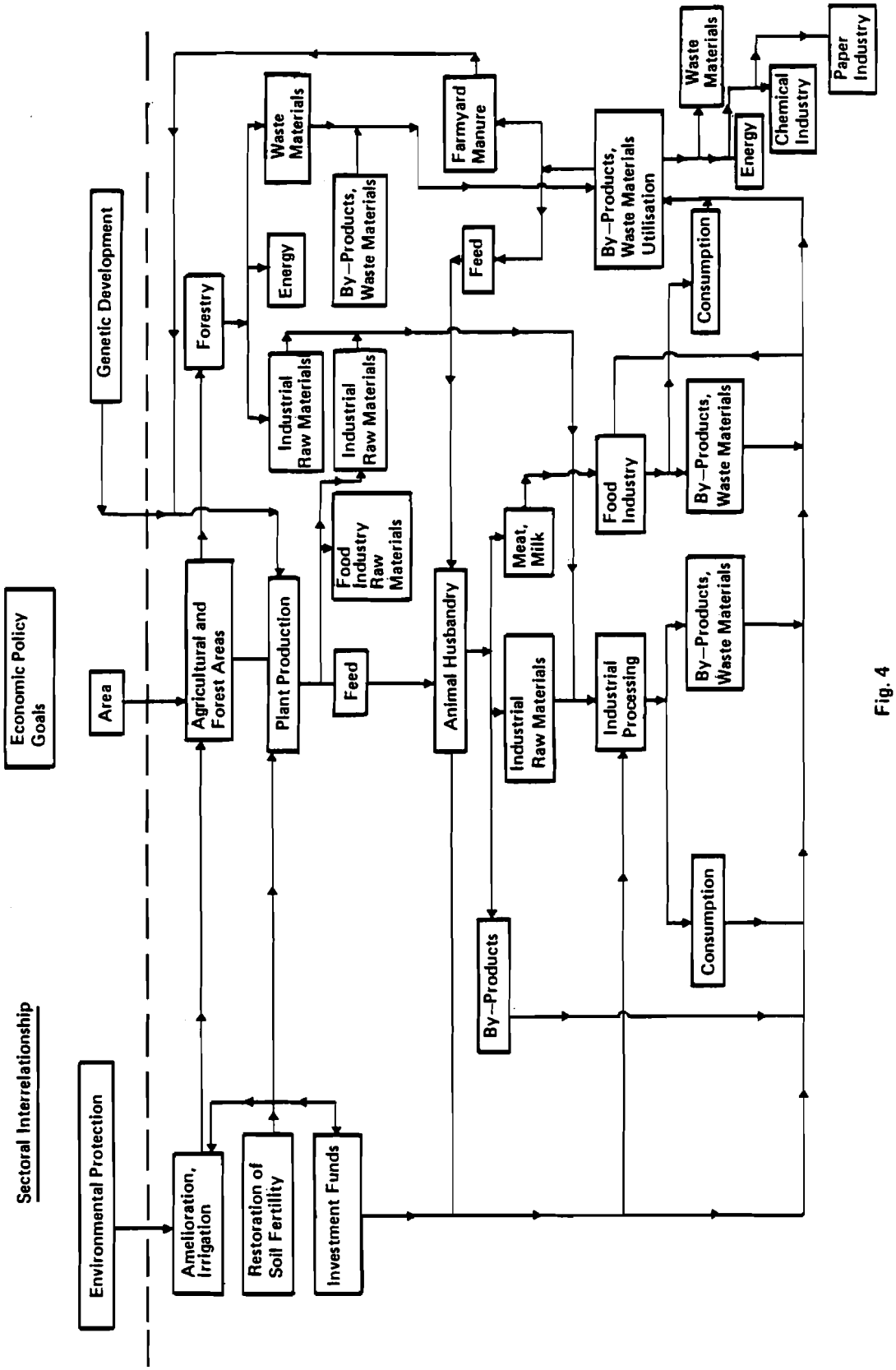
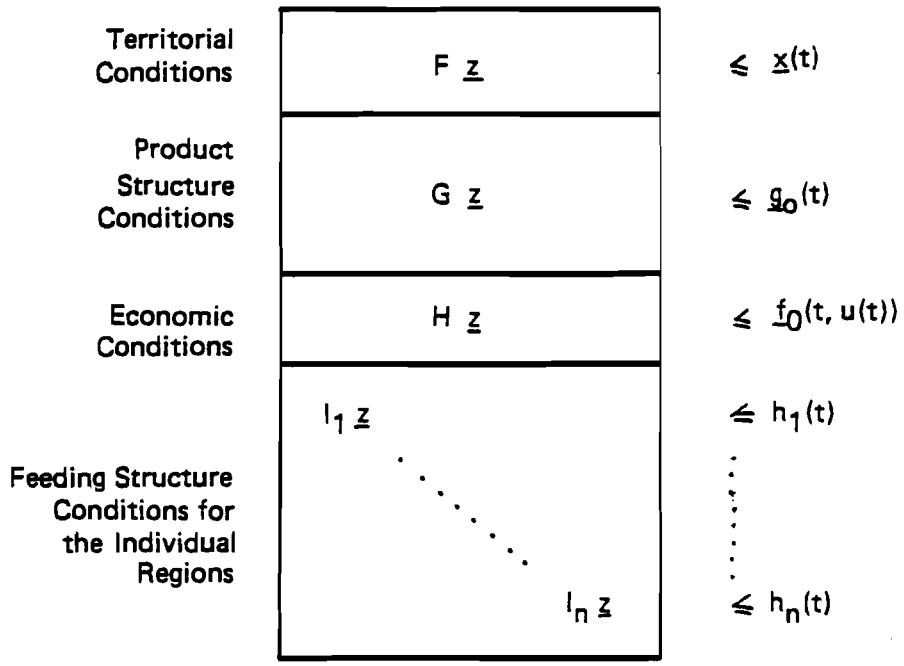

Sectoral Interrelationship
Economic Policy Goals
Genetic Development
Environmental Protection
Area
Agricultural and Forest Areas
Plant Production
Feed
Food Industry Raw Materials
Animal Husbandry
Meat, Milk
By–Products
Industrial Raw Materials
Industrial Processing
Food Industry
By–Products, Waste Materials
Consumption
By–Products, Waste Materials Utilisation
Energy
Chemical Industry
Paper Industry
Waste Materials
Farmyard Manure
Feed
Forestry
Energy
Waste Materials
By–Products, Waste Materials
Industrial Raw Materials
Industrial Raw Materials
Amelioration, Irrigation
Restoration of Soil Fertility
Investment Funds

Fig. 4

Production Model



Goal Function: $f(\underline{y}(1), \ldots\ldots, \underline{y}(T)) \longrightarrow$ opt.

Where $y(t)$ indicates the Product structure in the t–th Period

the Relationship between the Various Periods is Defined by

$(\underline{x}(t), \underline{z}(t)) \longrightarrow x(t+1)$ and by the Goal Function

Fig. 5.

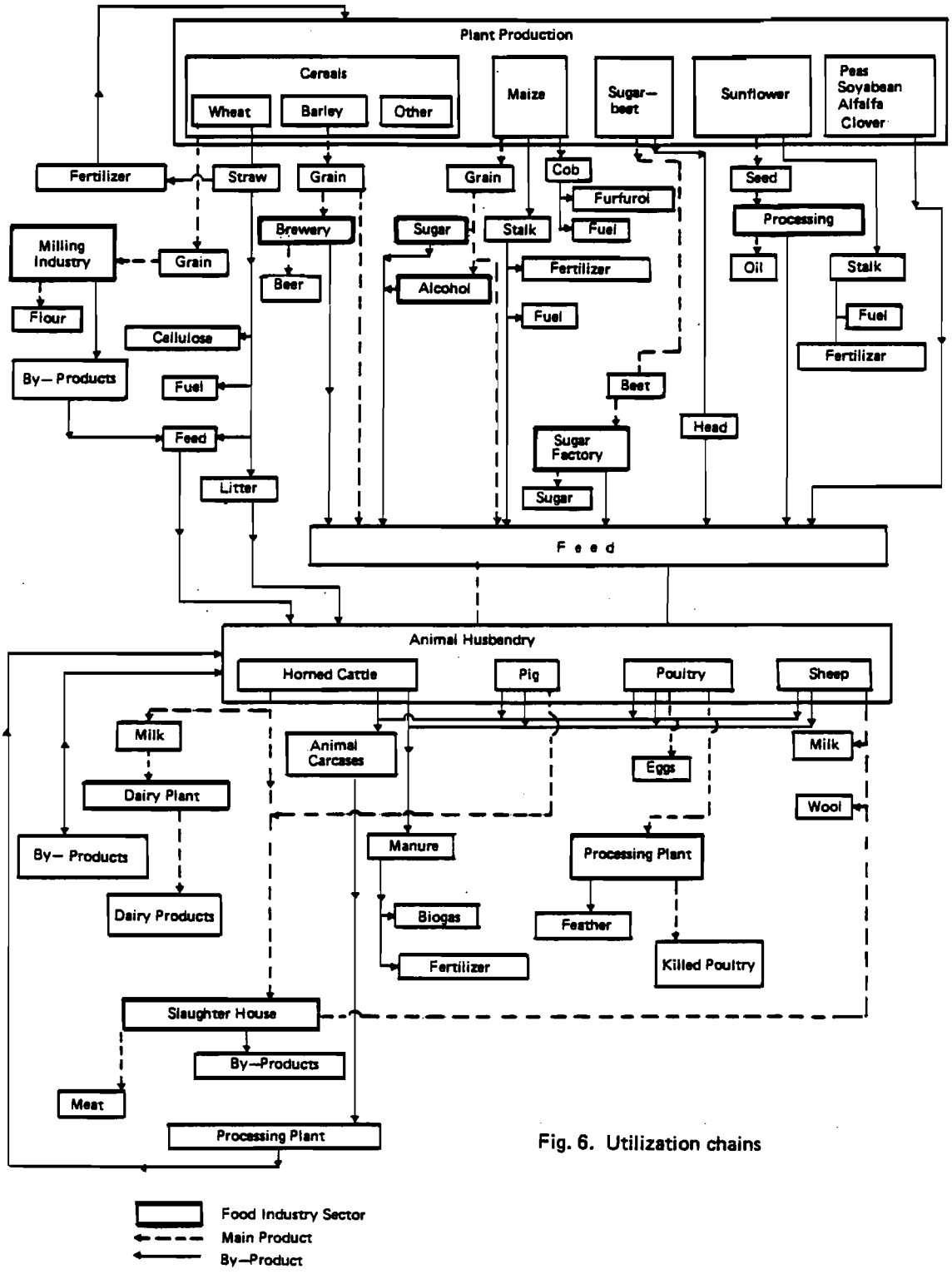


Fig. 6. Utilization chains

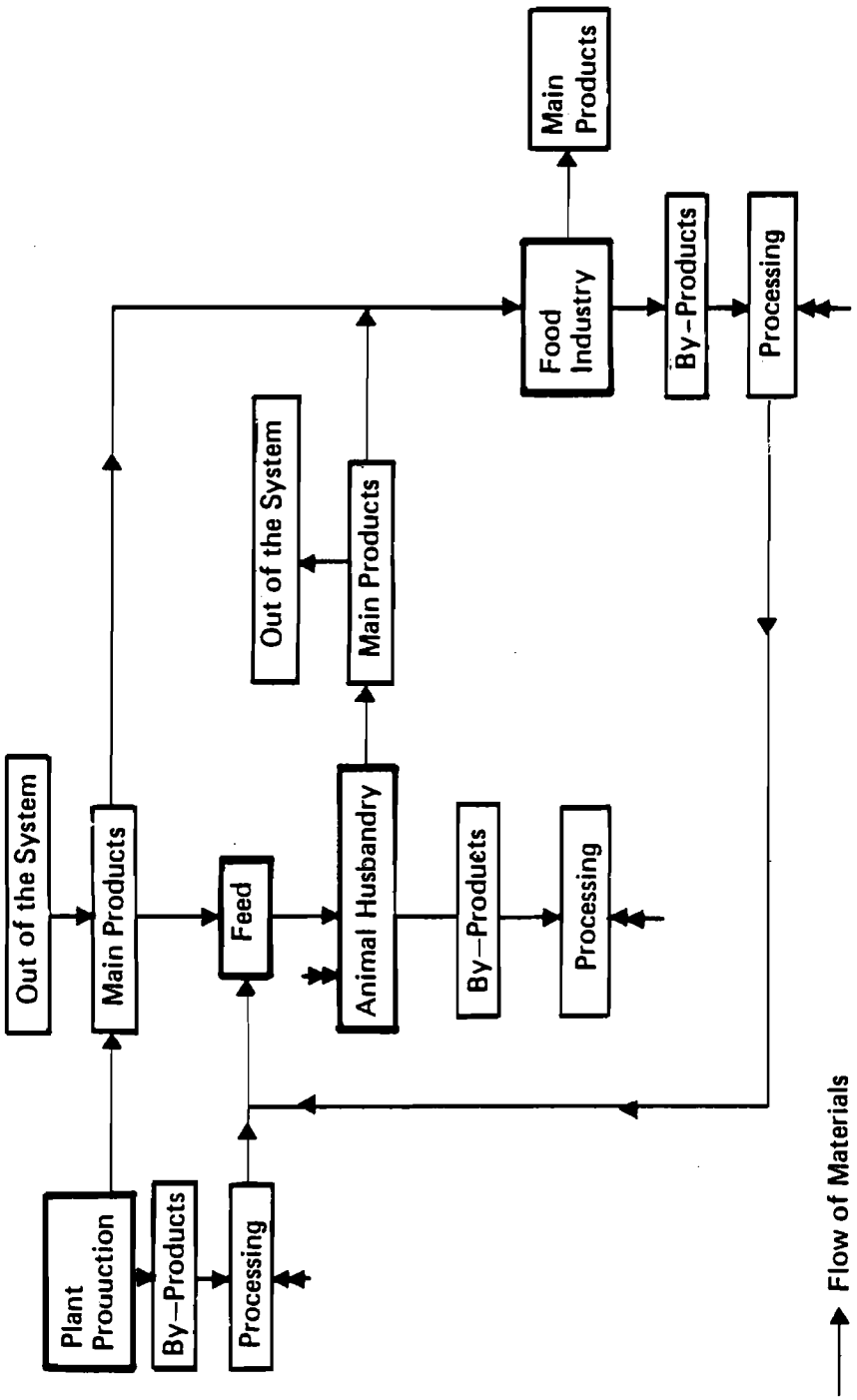


Fig. 7. Schematic representation of the model

NON-PHOTOSYNTHETIC SOURCES OF SINGLE CELL PROTEIN – THEIR SAFETY AND NUTRITIONAL VALUE FOR HUMAN CONSUMPTION

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1. Introduction

The first use of the name Single-Cell Protein and the first international conference on this subject was held at Massachusetts Institute of Technology in October, 1967 (Mateles and Tannenbaum (Eds.), 1968). In 1970 a Working Group on Single-Cell Protein (SCP) was first convened in Marseille by the Protein Advisory Group of the UN System (the PAG). In the subsequent four years this working group developed a series of guidelines, (PAG Guideline No. 6, 1983) for Pre-Clinical Testing of Novel Sources of Protein, (PAG Guideline No. 7, 1983) for Human Testing of Supplementary Food Mixtures, (PAG Guideline No. 12, 1983) on the Production of Single-Cell Protein for Human Consumption, and (PAG Guideline No. 15, 1983) on Nutritional and Safety Aspects of Novel Protein Sources for Animal Feeding.

These have recently been revised by a joint working group of representatives of the International Union of Food Science and Technology (IUFoST), and the International Union of Pure and Applied Chemistry (IUPAC) convened by the United Nations University (UNU) and are being reissued in its Food and Nutrition Bulletin. The modifications of Nos. 6, 12 and 15 are minor, but Guideline 7 is being completely rewritten because of the frequency with which gastrointestinal and cutaneous reactions of an apparently allergic nature have been observed with the feeding of RNA-reduced yeast and bacteria.

A 1972 symposium held in Aix-en-Provence brought together information on many of the toxicological, psycho-social, physiological and nutritional factors associated with the use of SCP materials grown on normal alkanes and gas oil and provided convincing evidence of the safety and nutritional value of the material for animal feeding and, when properly processed, its possible use for human feeding (Gounelle de Pontanel, 1972). By the time of a PAG symposium held in Brussels, Belgium in March, 1976, three European SCP products were considered ready for human feeding trials: Toprina (British Petroleum, Ltd.), Pruteen (Imperial Chemical Industries, Ltd.), and Liquipron (Liquichimica Biosintesis).

Each had been tested sucessfully in multiple species of experimental and farm animals with no evidence of toxicity. It was concluded that there were no scientific grounds for not approving these SCPs for use in animal feeding. However, questions continued to be raised about the safety for human consumption of the resulting animal products. These were based on the increased quantities

of paraffins and of odd-numbered carbon chain fatty acids found in some of them.

These issues were resolved by the data presented at a symposium convened under the auspices of the PAG in Milan, Italy, in 1977 (Garattini, Palgialunga, Scrimshaw (eds.), 1979). At this meeting, very extensive data were presented to indicate that odd-numbered carbon chain fatty acids are utilized through normal metabolic pathways and are modified at the same rate as even-carbon chain fatty acids, even when fed under conditions of fasting or stress. Moreover, tissue mitochondria of animals fed SCPs containing significant levels of odd-numbered carbon chain fatty acids responded in the same manner as those of control animals. The actual level of residual paraffins of chain length C14-C18, found in some hydrocarbon-grown SCPs, proved lower than that in many common foods in the U.S. and Europe. Moreover, they were conclusively demonstrated to have no effect on the viability and respiratory function of cells and they disappeared from tissues in a normal manner when their ingestion was terminated. Extensive successful nutritional and toxicological studies on both experimental and farm animals in the USSR were also reported at this meeting by R.S. Rychkov and G.K. Skryabin (Rychkov and Skryabin, 1981), together with additional studies in Japan described by M. Fujimaki (1981), and in Europe cited by D.A. Stringer (1981). After this 1977 meeting, there could no longer be any doubt that a number of SCPs could be safely and effectively fed to farm animals and their products consumed by humans.

2. The Significance of Nucleic Acid in SCP

At the time of the original 1967 Conference at MIT there was no general appreciation of the significance of the relatively high nucleic acid content of SCPs. In our own studies, in which yeast was consumed in addition to the usual diet of the individual at levels of 45 to 135 grams, we observed highly significant increases in serum and urine uric acid in subjects consuming the yeast. The increase occurred because uric acid is the metabolic end-product of the purine component of nucleic acid and it is relatively insoluble. At high serum levels and under certain conditions, uric acid crystals precipitate out in the joint, causing gouty arthritis, in the skin as tophi, or in the kidneys to produce urinary calculi. Among mammals, only man and the higher apes lack the enzyme uricase that breaks uric acid to the more soluble metabolic end-product allantoin. Thus, the nucleic acid content of SCP has no significance for animal feeding.

In order to determine more precisely the effect of the high nucleic acid content of yeast on serum and uric acid levels, we fed known levels of yeast for nine days to individuals on a constant formula diet (Edozien, Udo, Young and Scrimshaw, 1970). The results were a linear increase from 4.5 to 8.9 mg of uric acid per dl. at levels of intake from 0 to 90 gms of yeast per day corresponding to about 5.5gms of nucleic acid. Corresponding figures for urine were 510 $\pm$ 31 to 1853 $\pm$ 121. Based on these and other data, the PAG at a meeting in Moscow 1971 established a safe practical limit of 2 gms of additional nucleic acid per day from SCP's in addition to that in the usual mixed diet. This level was reaffirmed by a PAG working group of distinguished clinicians meeting in Geneva in 1975 (PAG, 1975).

3. Use of Yeast as Food

The use of the yeast *Candida utilis* for direct animal consumption was first explored by Delbruck in 1910 (Delbrueck, 1910), and thousands of tons of this yeast were consumed in Europe and Japan during World Wars I and II as meat substitutes and meat extenders. The term "food yeast" was apparently coined

by the British Royal Society in 1941 to center attention on *Candida utilis* yeast grown expressly for human consumption and to set it apart from yeast obtained as a by-product of the brewing industry (Medical Res. Council of G.B., 1945). It has been produced in a number of countries on substrates of both sulfite liquor from processing wood pulp and molasses from beets or sugarcane.

Apart from this war-time use, food yeast has not been consumed as more than a minor ingredient in processed foods for its functional properties or as a vitamin source. *Candida utilis* is on the GRAS (Generally Regarded as Safe) list of the US Food and Drug Administration (FDA), and is used in many processed foods in North America, Europe, Japan and throughout the world.

Because of the GRAS list status of *Candida utilis*, we had not hesitated in 1976 to feed 90 grams of Lake States *Candida utilis* per day for 11 days to 12 subjects. An unexpected finding in 8 of these subjects was the development of a mild papular rash on the palms and soles, followed by peeling of the skin (Young and Scrimshaw, 1975). This was painless and disappeared spontaneously when the yeast feeding was discontinued, or even before. In one of the cases, the rash extended to the dorsum of the hands and the penis.

Different batches of the same material were also fed at levels of 45 grams and 135 grams for a planned 10-week period. One of the subjects taking 135 grams/day developed a rash within five days that extended to the arms, back, chest, abdomen, and thighs. It was neither painful nor pruritic. In this case yeast consumption was discontinued on the seventh day, and after three days the rash had disappeared. This subject was then put back on a dose of 45 grams per day to complete the study without any recurrence of the lesion. Two other subjects fed 135 grams per day developed rashes on the legs and thighs with some itching at onset. Their yeast intake was not discontinued and the rash cleared without treatment on the fourth day. In none of the three was desquamation observed.

The cause of these reactions was never determined because the manufacturer was unwilling to cooperate and funds were not available to investigate the problem further. Since the material was on the commercial market with no reported problems from its use at lower levels as a food additive, we subsequently fed this yeast at a much lower level as a control in our investigation of SCPs produced on hydrocarbon substrates with no problems for the next ten years. In the fall of 1979, therefore, we were very surprised by a group of 28 "control subjects" receiving a commercially available batch of the sulfite-grown Lake States yeast (Scrimshaw and Dillon, 1981). Two weeks after beginning a double-blind trial it had to be discontinued because of adverse clinical reactions. When the code was broken, the principal problem was with the "control" material. One individual had a papular rash on the palms and soles and five individuals had gastrointestinal symptoms that included some degree of nausea, vomiting, and diarrhea. The manufacturer was uncooperative in supplying any helpful information, and no follow-up has been possible. It seems clear, however, that some difference in processing or quality control must have occurred.

4. Experience with Other Yeasts

In 1970 we tested for 90 days an RNA reduced yeast of a different species grown on ethanol with 50 subjects. None of them developed a syndrome of nausea, vomiting and diarrhea (NVD) that began about the third week. The remaining 41 subjects completed the study uneventfully.

In 1973, we observed a similar NVD reaction in 7 of 100 subjects fed 20 gms daily of *Candida tropicalis* grown on N-alkanes (Scrimshaw and Udall, 1981). The remaining 93 consumed the material uneventfully for 60 days. Acid washing

removed the offending substance in a batch fed to 56 subjects for 14-72 days, but reactions began to appear when a second batch of the material was fed. Presumably there was a critical difference in the processing of the two batches that was never identified. Solvent-extracted RNA reduced *Candida- lipolytica* grown on gas oil at Lavera, France was fed at a level of 20 gms per day in 50 subjects for 60 days with no adverse clinical reactions.

In 1975 we tested an RNA-reduced *Candida utilis* for the first time (Scrimshaw and Dillon, 1979). The original yeast, produced on beet molasses in Europe, was and still is in widespread commercial food use. Eight subjects fed 50-60 gms per day completed an eleven-day balanced study uneventfully. However, when 13 subjects were fed 35 gms of the material per day, a proposed 30 day trial had to be aborted after 10 days because of skin rashes in 3 of the subjects. Investigation revealed that to lower the cost the temperature of the RNA reduction process had been lowered from 140^0 to 80^0C and the treatment time slightly lengthened. When the trial was repeated in 51 subjects for 30 days with material processed at 140^0 to reduce the RNA, no problems were encountered.

Human tolerance to consumption of new single-cell protein from a methanol-grown yeast of the sub-family *Saccharamycoideae* as a significant dietary protein source was tested beginning in 1977. In initial tolerance testing studies, the whole cell preparation of this SCP caused nausea, vomiting, and diarrhea or skin rashes in 17% of the exposed subjects. Both types of adverse responses appeared to be allergic in origin. Subsequent process variants of this material proved more acceptable (Scrimshaw and Udall, 1981).

Nearly a dozen other authors have described feeding various quantities of *Candida utilis* in amounts up to more than 100 grams per day to human subjects without any adverse gastrointestinal effects, but others have described different experiences. For example, Goyco, Santiago and Rivera (1959), reported unacceptable gastrointestinal reactions in subjects receiving as little as 15 grams per day of molasses-grown Torula yeast in Puerto Rico. Retrospectively, it was suspected that the defoaming agent employed was the cause, but this has never been confirmed. The subjects fed a food yeast in an unpublished U.S. Army field trial during World War II experienced no difficulties during the first 30 days, but then developed so much gastrointestinal discomfort, possibly a result of accidental contamination, that the study had to be terminated.

5. Experience with Bacteria

In 1971 we had our first experience in feeding bacteria to human subjects, 20 gm per day of an RNA reduced, *Acinetobacter coaceticus* for 90 days. Eight of 50 subjects developed an NVD syndrome similar to that observed above with the yeast grown on ethanol. When the same material was acid washed and then fed to another 51 subjects for 30 days, no adverse reactions were observed. We have recently begun testing bacteria grown on methanol but the results are not yet available.

Consumption of other washed, sterilized bacteria has been reported to cause severe gastrointestinal disturbances in humans. (Waslien, 1975). Ingestion of 25-50 g of *Hydrogenomonas eutropha* cells resulted in diarrhea, headache, gastric distress, and weakness in five subjects within 24 hours, and *Aerobacter aerogenes* ingestion caused similar symptoms (Waslien, Calloway and Margen, 1969). It is clear that the direct use of microbial cells as food for humans will require more study and development.

6. Fillamentous Microfungus

In 1977 we fed 20 gm per day of a fillamentous microfungus grown on starch to 100 subjects for 30 days in a double blind trial. Rank-Hovis-McDougall provided identical tasting cookies, with and without the test material. The trial was uneventful. More recently we have fed 10 grams daily of "Pekilo Protein", a fillamentous microfungus *(Paecilomyces varioti)* grown on sulfite waste liquor to a total of 50 subjects for 30 days. Half consumed the test material and the other a placebo for 25 days. The administration was then reversed in a double blind crossover study. This trail also proceeded uneventfully, and the material proved acceptable and well tolerated. I was very pleased to learn that Prof. Torev in Plovdiv has produced a fillamentous-fungal product that is well tolerated and palatable in human foods.

7. Nutritional Considerations

From our own laboratory we have obtained data from N-balance studies on several of these single cell protein products. By feeding them at a level of approximately 0.4 gms of protein per kg per day, digestibility can be reliably calculated. For the *Candida utilis* grown on sulfite (Lakes State Yeast Co.) digestibility was 78%. For RNA-reduced *Candida utilis* grown on beet molasses (Nestle Co.) it was 84%. For the Rank-Hovis-MacDougall fillamentous microfungus mentioned previously it was 75%. Digestibility figures for these and other SCP's reported in the scientific literature are given in Table 1, (Waslien, Calloway, Margen and Costa, 1970).

Table 1. Some nutritional value data on single-cell proteins for human subjects.

Material	N	Digesti-bility	NPU	Reference
Yeast:				
Candida utilis	7	83*	58*	Waslien et al. (1970)
Sub-family *saccharomycoideae*	6	84	54	Scrimshaw & Udall (1981)
Candida utilis	7	84	48	Scrimshaw (unpublished)
Fungi:				
Fusarium graminearum	13	78	65	Udall et al. (unpublished)
Fusarium graminearum + 1% Methionine	6	79	73	Udall et al. (unpublished)
Paecilomyces varioti	50	81	54	Udall et al. (unpublished)
Algae:				
Chlorella pyrenoidosa	6	66	--	Lee et al. (J. Nutr. 92: 281, 1967)
Scenedemus obliquus	5	68	--	Dam et al. (J. Nutr. 86: 376, 1965)
Chlorella pyrenoidosa (low level)	5	57	--	Dam et al. (J. Nutr. 86: 376, 1965)
Chlorella pyrenoidosa (high level)	5	59	--	Dam et al. (J. Nutr. 86: 376, 1965)

* Corrected for endogenous N excretion

Data on amino acid scores and net protein utilization for many of these are available but are of limited value.

Because methionine is limiting in their amino acid pattern compared with that of the 1972 FAO/WHO reference amino acid pattern, amino acid scores ranging from 21% to 93% have been reported. When single cell proteins are fed at the level conventionally used for determination of Net Protein Utilization (NPU) to human adults (approximately 0.4 gm of protein per kg) or to rats diets with 10% protein, methionine addition improves N retention and rat growth. However, when single cell and legume proteins are fed at levels adequate for N balance, sulphur amino acids are far less limiting and the NPU is greater than predicted from conventional assays, (Scrimshaw and Young, 1979). This issue has been reviewed in two recent publications (Pellett and Young, 1980) and (Bodwell, Adkins and Hopkins (eds), 1981). It is sufficient to say in this review that nutritional value is not the limiting factor in the utilization of well produced SCP's in processed foods and mixed diets. The limitation will be due to issues of palatability, tolerance, functional properties and cost.

8. Preclinical Evaluation of SCP

All of the SCP products we have tested on human subjects have undergone extensive preclinical testing in experimental animals as indicated in PAG guideline No. 6 and must have also been extensively and successfully fed to farm animals, including chicken, swine, and calves. In addition, those grown on petroleum hydrocarbons must have been produced in accord with PAG Guideline No 12. However, with increasing experience we have gradually evolved procedures for clinical testing that are a marked improvement on this in PAG Guideline 7.

Among the key provisions will be the requirement for a tolerance test conducted on a double-blind crossover basis with at least 50 subjects. The design calls for 25 experimental and 25 control subjects for 30 days, a short break and then reversal of the two groups. A battery of biochemical tests indicative of liver and kidney function is obtained at the start and at the end of a 30-day period. The level at which the test material is fed should be at the upper range of intended use, at least 15 to 30 grams per day.

Since adverse reactions due to infections and other causes unrelated to the material fed, are not uncommon in any extended feeding study even of such accepted foods as milk or soy, the problem is to distinguish those responses that are due to factors unrelated to the test protein from those that are due to specific intolerance to it. Without an adequate control group this is impossible when the frequency of adverse reactions is low. Of course, when the frequency is greater than 10% or the symptoms are as distinctive as those of the NVD syndrome or the eruptions on the palms of the hand and soles of the feet, described above, the task is easier.

If successful, tolerance trials should be followed by a N- balance study in 6-8 subjects in order to obtain data on digestibility and an estimate of net protein utilization. Any adverse reactions call for modification of the processing procedures in an attempt to neutralize or eliminate the substances responsible. Ideally it would be desirable to determine whether or not this has been achieved without the need to expose additional subjects to this risk of reaction. This is sometimes possible as described below.

9. *In vitro* Approaches to SCP Tolerance

It should be stressed that no amount of preclinical testing in experimental and farm animals can eliminate the possibility of intolerance when an SCP product is fed to human subjects. Animal trials can eliminate true toxicity and make human trials ethical, but they cannot predict the frequency of allergic reactions of a gastrointestinal or cutaneous nature at an unacceptably high level of frequency or severity. Even for subjects who have experienced an allergic reaction neither skin tests with extracts of the material fed nor the radio immune test (RIST) for circulating antibodies to the test material have not thus far yielded consistent or useful results. However, it has proved possible to screen processing variants by incubating extracts of them with cultures of lymphocytes taken from individuals showing such allergic response to the original material. As mentioned above a *Candida utilis* grown on beet molasses and RNA reduced at 80^{0}C caused a high frequency of skin reactions of an apparently allergic nature in human subjects. Not only did an extract of this material stimulate the replication of lymphocytes from reacting individuals but also it was possible to fractionate the extract and demonstrate that the response was due to a protein with a molecular weight of approximately 55,000 Daltons. RNA reduction at 140^0 eliminated both the *in vitro* and *in vivo* response. One limitation to this technique is that the responsiveness of the lymphocytes is gradually lost after a few months. Since then *in vitro* lymphocyte response to the methanol grown yeast preparation mentioned previously was assessed by the lymphocyte transformation test (LTT). The test gave positive results in sensitive subjects, which were two or more-fold higher than those in age-matched control subjects. The effect was specific and reproducible provided the tests were conducted with (i) subjects who were sensitized not more than three months ago and (ii) control subjects did not have a positive LTT response to Monilia antigen, in which case there would be a cross LTT response with the methanol-grown yeast. Because of its specificity, the LTT can be considered a useful aid in diagnosis of human allegic response to consumption of the methanol-grown yeast.

When applied to provide an *in vitro* indication of potential allergenicity in industrially modified preparations of the methanol-grown yeast, the LTT successfully identified heat shock at an acidic pH as a process that could attenuate the factor(s) responsible for gastrointestinal symptoms. We have since further refined this method and are applying it to additional SCP products that have caused apparently allergic reactions.

In Summary:

Selected yeasts, bacteria and fillamentous microfungi can be used as significant protein sources in human diets but processing to reduce their RNA content is required when they are to be used in the quantities necessary for this purpos- Allergic responses have not been described with fillamentous microfungi but have proved common with yeasts and bacteria. Fortunately this problem can usually be overcome by suitable processing. Although thus far experimental animals have proved of no value in detecting potential allergenicity for humans, lymphocytes of sensitized individuals can be used to screen processing variables for improved tolerance. Human feeding trials must be preceded by extensive preclinical testing in experimental and farm animals in accord with the PAG guidelines.

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USE OF NON-CONVENTIONAL PROTEIN IN FOOD PROCESSING

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1. Introduction

World nutrition balances prepared by FAO permanently show malnutrition of the population in the developing countries and a simultaneous growth of satiety in the developed countries. Food consumption estimated in calories per day in the countries of the Far East and Pacific amounts to less than 2000 kcal with 2200 kcal the minimum demand, while in the developed countries it exceeds 3100 kcal, i.e. Poland 3500 kcal.

In many developing countries the area under cultivation could be doubled, and in all the countries there are possibilities for considerable growth of agricultural production and for decreasing losses following the harvest and those which occur during food processing and storage. Actually, on a world scale, we have the potential for producing a much greater volume of food per capita than 10 years ago, and an incomparably greater one than 100 years ago. The present-day total food shortage is, primarily, a consequence of the weak economy of developing countries, irrational distribution and unfair allocation of food reserves. This results mainly from political and not agricultural and technical conditions. The way to improve the situation is not hopeless although the essential problem of the actual fight against hunger lies in the level of people's income, which depends on the socio-economic advance of single world regions (Rutkowski, 1979)

2. Demand for Food Protein

Both in developed and developing countries, the protein deficiency as basic food element, is a major topic of discussion. Actually, apart from the tropical belt there is no deficiency of protein in the physiological sense (Mauron, 1978). Though there is certainly one (kwashiorkor), in some regions of the world where the diet is extremely unbalanced and the economic standard of the population is particularly low. In developed countries the shortage of edible protein in nutrition of even poor social strata occurs rarely, and the level of its consumption is determined by prices of food articles of animal origin. Therefore, the phenomenon of protein shortage can be considered, in general, as:-

- the *direct* effect, where plant protein constitutes the basic element of food, and the energy needs of the body are covered two thirds by direct consumption of pulse and grain products. This situation occurs, more or less intensified, in all developing countries;

- the *indirect* effect, where plant products (without oils) cover only one-third of the energy demand. An inhabitant of these countries, developed ones as a rule, consumes, on the average, four times more meat and fats and nearly six times more milk and eggs than an inhabitant of developing countries. This is also reflected in the fact that, for example, the population of the USA and Canada as well as of many European countries consumes directly less than 10 per cent of grains, since over 90 per cent of grains are used for fodder in order to produce an adequate amount of meat, milk and eggs (Rutkowski & Kozlowski, 1981)

Thus, the problem of protein shortage in developed countries consists, primarily, in lack of fodder protein which is the raw material for animal protein production. It must be realized that the conversion of plant protein into animal protein is extremely costly, and losses in this process are quite substantial. For example, protein losses in the production of poultry meat protein (broilers) amount to about 70-80 per cent, while in beef production they are as large as 90-96 per cent.

Under these circumstances, the consumption of animal protein exceeding man's physiological demand (about 0.5 g/1 kg weight/day) is an extravagance, which becomes ever more common with the growing incomes of a population. No less important are customs of the people based on a belief that consumption of meat dishes is a mark of affluence.

The problem of protein shortage in developing countries where the consumption of animal products is not as high as in developed countries, resolves itself into the correlation with food consumption in general. Though in the future, with the growing living standard of the population, there is to be expected in those countries also an increased consumption of animal products. A higher demand for these products leads to a general increase in the cost of food, and depends entirely on cheap food deficient in protein. It may be anticipated that this tendency will continue in the future, but we hope that it will not reach such high levels as in Europe or in the USA, both for climatic, religious and custom reasons.

Growing consumption of meat and meat products, observed most markedly in a number of countries in recent years, expands the disproportion between plant protein production and the growing demand for animal products. The point is, how to counteract the increasing shortage of fooder and the growing consumer demand for bigger production of non ruminant meat and meat products. Of course, the prior task is to increase production of conventional fodder protein and its optimum use. No less important is the use of a possibly higher share of the produced animal protein for edible purposes. This alone will not do, however. The decreasing area of arable land in developed countries and the growth of agricultural production costs with the rising output, focus the interests of science on new methods of obtaining and processing non-conventional proteins products (NCP). The prior ones among these are the following:

- chemical synthesis of protein-like substances and their use as fodder or food,
- microbial synthesis of protein for fodder concentrate for pig and poultry from raw materials useless in human nutrition,
- replacing a part of animal proteins by plant proteins in human and animal nutrition.

Unfortunately, much of this NCP exists essentially as inert powders, lacking any functional properties such as water-binding or the capability of being

texturized. Their only use has been as inert additives to simple materials. SCP, algal protein, leaf protein and fish protein concentrate have not become realities. As a general rule, these foods are organoleptically unacceptable, and therefore studies should be made of ways to preserve the native protein or to incorporate it functionally into the processed protein (Mark, 1979).

3. Non Conventional Protein

3.1. Synthetic Protein

Protein synthesis has long been a point of scientific interest. The attempts undertaken by Fischer in the early twentieth century to reproduce the protein structure from amino-acid substances initiated a systematic research in this field. A certain success was reached in the so-called pansynthesis of amino-acids and their condensation in protein particles. The works of Miller, and later those of K. Stewart and S.W. Fox, led to obtaining a protenoid containing amino-acids connected by peptide linkage, the molecular weight of the protenoid amounting to about 8000. Thus, considerable success was achieved aiming at a synthesis, and, with the present state of knowledge and technique, it can be assumed that a synthesis of a protein-like product of adequate amino-acid composition is realistic, similarly to the synthesis of fats - glycerides. From initial components thus obtained, products corresponding to natural ones can be composed. In this way a proverbial "synthetic cutlet" can be made. But why do it? A synthesis of meat through animal organism is and will be for a long time, after all, the cheapest means of production, and now it is impossible to say when synthetic products will come up to meat in their sensoric quality (Rutkowski 1979).

Another method for producing novel biosynthetic food protein products is the so-called plastein reaction. By this way from low quality proteins full nutritive value protein preparations will probably be obtained soon. However, it is necessary to technically master the plastein reaction, a process has been elaborated on a laboratory scale by M. Fumjimaki in Japan. It must be assumed that obtaining an adequately cheap product, will still require persistent and arduous work (Rutkowski 1981).

3.2. Single Cell Protein (SCP)

Microbial biosynthesis of proteins is the oldest and widest field of research. For over 100 years researchers have been fascinated by the possibility of involving micro-organisms in producing from waste substances proteinous biomass (BMP), or single cell protein (SCP) when the micro-organisms are harvested and separated from the substrate. The world is frequently thrilled by the successful results of laboratory research. In studies it is assumed that from 100 g of carbohydrate substrate the maximum practical yield of true protein by bacteria is 35g and by yeast and fungi 25g (Worgan, 1972). By an other calculation 1kg of barley flour supplemented with an inorganic nitrogen source would give about 500g d.w. of meat-like mycelium containing 150-200g high quality protein. The same amount of barley used as pig feed would yield only 15-20g of meat protein (von Hofsten, 1976). However, few of these methods are used on an industrial scale, either for economic reasons or because of difficult technical solutions of the equipment for processing. Successive achievements in this field were the following:

- 80 years ago the industrial production of Torula yeast SCP in Germany,

- 60 years ago the use of waste-sulphite liquor and wood waste in SCP production,
- 20 years ago taking up of the production of yeast SCP on n-paraffins of crude oil,
- 10 years ago presentation of edible bacteria SCP produced on a technical scale, obtained from methane.

The production of baker's yeast on conventional substrates such as molasses played only a minor role as a protein source in human nutrition. It must be recognized that there is a strong tendency for the production of SCP by cultivating yeast on hydrocarbons and waste. The best-known are *Candida* cultivated on a hydrocarbon substrate for use as a protein rich component of animal feeds. As important is research done on recycled whey lactose, and effluents from potato, cassava, and maize starch factory into food protein by yeast. Much attention is given to converting cellulose by cellulases into glucose, which can be converted to alcohol and yeast protein. In general the crude protein content of yeasts lies between 50 and 65%.

Another route for the production of SCP is the bacterial cultivation on purified paraffins, methanol *(Pseudomanas)* or on ethanol *(Acinetobacter-Nestle)* followed by removal of nucleic acid from the product, to give suitable products for use in human consumption (Mauron, 1978).

The crude protein content in bacteria SCP lies between 67-80% but they contain twice as much nucleic acid (15%) as yeasts.

From a nutritional viewpoint high ribonucleic acid (RNA) content of SCP constitutes a problem since humans are unable to degrade the breakdown products of purines and pyrimidines. For this reason SCP is considered more as a feed-supplement. The amino-acid profile of SCP also shows some deficiences and supplementation with cysteine and methionine would be probably necessary (Rogers, 1978).

Most SCP enterprises have been concerned primarily with the production of animal feed protein. It is accepted that the animals can, with certain types of components (potentially toxic to man) serve as a "filter". On the other hand, some lipid (i.e. branched-chain or odd-numbered fatty acids) and other components (i.e. polynuclear hydrocarbons) in the feed may accumulate in the animal tissues. Much of the experimental work shows that under suitable production extraction and purification conditions protein feeds can be manufactured which pass safety tests (Aylward, 1979).

At present prospects for SCP intended for animal feed appear to be decidedly rosy. The price may be expected to fluctuate rather rapidly and settle down to lie between that for soya meal and that for fish meal. On the contrary after the first enthusiasm for the direct use of SCP in human nutrition, it soon became evident that bacteria, and even, to a somewhat lesser degree, yeasts, could not be used as a protein source for human beings unless their content of nucleic acid was considerably reduced. Nucleic acid highest permissible daily dose is 2g. This intake can be sustained for a long time without elevation of the uric acid in the blood (Mauron, 1978).

The use of yeast and bacterial protein for human nutrition require complete removal of substances which lower its nutritive value and to give the product suitable organoleptic qualities. It is necessary to solve the simple industrial technology of separating protein from the cell, removing substances such as nucleic acids (6-18%) and odd fatty acids (fat content 5-10%) and other unspecified flavour factors.

It is often assumed that small scale SCP production can easily be made operational. This can be considered a serious underestimation of the problems involved. Positive results are more likely to be achieved in larger (ca. 100,000 ton SCP/year) industrial scale process.

In so far as considerable success is to be noted in using yeast BMP as animal feeding stuff the concepts of further advance in this field are considered realistic.

3.3. Algal Protein

From ancient times algae have formed a part of the diet in certain civilizations (Asia - Japan, Africa - Chad, America - Aztec). Simultaneously, with or without success, research works are continued on economic and technically realistic use for the production of the biomass of algae *(Chlorella, Scendesmus and Spirulina)* which have aroused the world several times as the potential source of protein (Mauron, 1976).

The great importance of algae is that they possess the advantageous property of growing in a strictly inorganic medium. Propagation of algae can be carried out in a closed system (fermenters) or in open ponds or lagoons. Algae contain between 50% and 65% protein and nucleic acid content about 4%. The EEA content is similar to that of plant protein. The PER-value lies between 2.0 and 2.8; NPU ca. 60 (Priestley, 1976).

It is true that sophisticated systems for culturing micro-organisms from inorganic materials, such as those algal systems which depend upon artificial illumination and rapid pumping of the algal suspension are unlikely to be used. Algal systems which utilize sunlight as an energy source are in a stronger position, but practical application on a large scale will remain dependent upon reducing the technology required to very simple terms and diminishing the capital requirements to levels appropriate to developing country economies (Szczepanik, 1977).

Algae cannot be produced economically on an autothropic basis using conventional SCP production techniques due to prohibitive energy costs. Cells must be fractionated in order to render the proteins available for direct human consumption, since the intestines do not possess the enzymes to break down the cell walls. While fractionation is easily achieved in the laboratory, extension to the production scale is fraught with difficulties and the cost of the final protein product could increase by at least sevenfold (Priestley, 1976).

3.4. Fungal Protein

Research on lignocellulolytic fungi are concerned with their use for the production of edible protein. Lignocellulolytic fungi that cause white-rot and other forms of decay in dead trees are able to hydrolyse lignocellulose. In Thai isolated fungi (IDRIC, 1981) that grow rapidly and increase the digestibility and microbial protein content of sugarcane bagasse, rice straw and other lignocellulosic waste used as fodder (BMP). Fungi are being selected that neither generate toxic materials nor present any hazard to human and animal health. The idea of use for food protein production in submerged liquid culture of *Polyporus squamosus* has been described by Prof. A.Torev from the Plovdiv Agricultural Academy, and *Sporotrichum pulverentum* by Dr. v. Hofsten from the University of Uppsala. The mycelial mass (2% d.w.) obtained can be dried by various conventional methods and used as a protein component (25-40% d.w.) in foods (v.Hofsten, 1976). Nutritional and toxicological testing of these products have still to be completed.

3.5. Plant Proteins

(Rutkowski, 1979; 1981; Rutkowski & Kozlowska 1981)

The conception of protein brings to mind meat, milk and eggs. Meanwhile, the share of plant protein in diets ranges from 30 per cent in the USA to 87 per cent in South Asia and West Africa. Difficulties encountered in increasing animal production, and primarily, the rising price of meat, called the attention in the past decade to the concept of replacing animal protein in food and fodder by adequately prepared vegetable protein. In considering this concept from the viewpoint of food policy it can be assumed that replacing each kilogram of meat by a substitute or analog of plant protein increase protein would food reserves five to tenfold. The essential thing for the average consumer is that he can purchase meat products of high nutritive value for a reasonable price.

The concept of obtaining meat-like products from plant proteins arose about 100 years ago when Kellog obtained gluten based products resembling meat dishes. However, only after World War II in view of worldwide economic difficulties and of the remarkable advance of research on soybean protein carried out over the last 40 years, soybean preparations became available on the food market. In the late 1950s protein concentrates and isolates were the first products, the moderate addition of which to meat products has been approved by consumers. Further progress was the introduction of textured vegetable protein in the 1960s. Although the production of textured products based on spun proteins has not realized hopes so far, mainly for economic reasons, the introduction of textured products based on flour and extruded soybean concentrates fully met the requirements for product substitutes of average quality. Undoubtedly, a further step is the introduction in the market of frozen fibrous protein isolate. This isolate is of a high grade and can be introduced in meat products in considerable amounts without lowering their quality.

The task during the 1970s was then is to master production and use of plant protein products (mainly of soybean) in human nutrition in the developed countries. Introduction of these products in the market was facilitated by the following:

- rise of meat and milk prices on world markets,
- dwindling hope of achieving considerable amounts of edible protein from sea resources and by microbial synthesis.

The expansion of the scope of using plant proteins as ingredients in meat products, baked goods, confectionary products and in beverages was determined by the favourable results of nutrition studies, and by defining their functional features, The issue in 1971 by the U.S. Department of Agriculture of a permit to use textured vegetable proteins as partial substitutes for meat in the lunch school programme also encouraged production and consumption. The range of using vegetable protein products, developed so far, includes the following:

- Improvement of functional properties of products such as achieving better bread toasting, better firmness of sausages, etc. For this purpose, small amounts of proteins of limited functional influence are added to the product (sausages 3-5%, bread 5-10%).
- Partial, up to 50%, replacement of the expensive and scarce meat in mass consumption articles, i.e. minced meat products, pates, hamburgers, ready-to-serve foods, etc.

- Enriching of food products with cheap vegetable protein in order to improve diet standard and supply better food to people in some world regions.
- Creating of new food articles such as coffee whiteners, confectionary creams, frozen desserts, beverages, sauces, meat and bacon analogs, and other products obtained solely from vegetable proteins.

At the present development stage of the technology it can be said that vegetable protein products are winning, step by step, an even stronger position on the food market. The way is not easy, and many drawbacks need to be overcome. Let us consider some of them.

Advances in the production of vegetable protein products is linked primarily with improving their functional and nutritive qualities.

The direction of developing optimum functional qualities depends on the destination of the preparation. That is why many types of products are available as, at the present stage of science, we are able to obtain a product adapted, to the highest degree, to technical requirements of the article. The best example are all kinds of modifications of vegetable protein isolates.

In order to ensure adequate functional qualities of vegetable protein additives, often more than one preparation has to be used. Such is the case with meat products. Roughly speaking, meat products are composed of:

- emulsifying/gelling proteins, the properties of which can be improved by the use of isolates, and of
- fibrous proteins, for the replacement of which products are needed of similar qualities to those of muscle tissue such as extruded proteins, frozen spun and spun protein fibers.

The aim is to improve structure, firmness and chewing properties of the products, high water-binding capacity (1:4 to 1:5), to maintain good gelling properties and emulsion stability regardless of the temperature and heat treatment applied, and also to improve the tolerance for medium pH and the presence of electrolytes present in it (e.g. salt and phosphates). Consequently, in producing vegetable protein products for the meat industry, the aim is to achieve products which give a good, low-viscosity in brine, strong after heating, good and smooth texture, firm binding with meat, good fat emulsification, good water-holding capacity also in cooked products, and primarily not causing colour deterioration or an off-flavour in the final product.

The problem of the nutritive value of vegetable protein products arouses discussion. The nutritive value of protein concentrates (PER about 2.2) and isolates (PER about 1.8) is fairly low. It can be raised in a relatively simple way by the addition of methionine and lysine or by a more complicated plastein reaction. However, this operation seems to be in use only in the case of preparations that are the sole protein element of the diet as, for example, in case of baby foods, low-calorie foods or food for diabetic patients. Our experiments with adding 20% and 40% of soybean isolates and concentrates to low - grade meat showed no essential lowering of the nutitive value of the mixture.

A specific problem is created by anti-nutitive factors present in soybean such as antitrypsins and hemagglutinins. We found also in this case, however, a high degree of their deactivation during processing. Thus, in evaluating the nutritive value of products containing them, their antiproteolytic action was not found in vivo when tested on rats.

A more complex problem to be solved is obtaining vegetable protein products completely free from the off flavours (beany grassy, etc.) as well as its reversion after dehydration and heating. Although the occurrence of the beany

flavour, particularly in soybean isolates and isolate by-products, has been largely eliminated, flavour remains to be the essential factor limiting the volume of soybean preparations added to food products.

A similar problem is caused by oligosaccharides *(stachyose)* present in flours, grits and concentrates which bring about the discomfort of flatulence. Many researchers believe that both the difficulties and high cost of eliminating the beany flavour and the flatulence factor, particularly from flours and concentrates may be a deterrent in their use.

Therefore, intensive research is being carried out to obtain vegetable protein products from seeds of cotton, groundnut, sunflower, rape, pea, triticale, etc.

Taking into consideration the opinions of food producers using vegetable protein products, and the results of many consumer surveys, a growing use of these products is to be expected in the near future for these products in the following fields:

- In meat processing an increased use of extruded flours and concentrates is expected in the form of meat substitutes in making products such as soyburgers, meat balls and cubes, meat pates, etc. Also the use of isolates as technological additives to improve the firmness of meat products and canned ham, to increase the water-retention capacity of the meat mass and the stabilization of fat-protein emulsions in processed articles such as cooked and cured sausages, luncheon meat, etc., is expected to expand.
- In the bakery industry the interest in vegetable protein products will grow since their addition improves toasting, extends shelf-life and allows the production of various quality types of bread.

A marked advance is expected in elaborating new types of all kinds of adequately modified vegetable protein isolates. They are ever more used in confectionary for producing whipping agents and whipped toppings, pastes, desserts and frozen desserts.

The possibility of obtaining, by modification of plant protein products of a high tolerance to water hardness and medium acidity, offers good possibilities for producing high-quality coffee whiteners, still-and carbonated- beverages, fruit (citrus, pineapple) and vegetable (tomato, carrot) juices, desserts with natural fruit flavours, sour candies, acid types of fillings, jams and jellies. In the group of beverages the use of modified isolates is feasible for foam stabilization in beer.

A great future in using all the types of vegetable protein products is foreseen in producing convenience foods, instant and canned soups, sauces, salad dressings, and cheese spreads. In these the preparations are a factor in enriching protein and giving suitable physical quality to the product, particularly by thickening.

Protein concentrates and isolates are used especially for protein enrichment or replacement in the production of baby foods, dietetic and geriatric foods. Isolates, owing to their low amount of non-protein calories, are suitable for production of low-calorie foods.

In the promotion of the use of vegetable protein products very much depends on the elaboration of new improved production technologies. Further development requires, primarily, removal of the beany flavour from soybean products, greyness from sunflower products, and the bitter taste from rapeseed products, etc. It must also be taken into account that in order to obtain good vegetable protein raw material, the traditional oil extraction technologies will be changed and a new type of processing will be introduced such as wet-milling of

oilseeds.

Future studies will expand our knowledge of physical and chemical properties of vegetable proteins. This will help to develop better technology for obtaining preparations of better and desired qualities. Already now we can influence the properties of the preparations by chemical (e.g. succination) by mechanical and heat treatments to give them desired functional qualities, and by the plastein reaction we can considerably raise their nutritive value.

3.6. Animal Waste Protein

The best use of animal protein is its direct consumption. Unfortunately some abattoir products, in several countries determined by food habits, religion and customs are rejected by human consumers. They are proteinaceus materials, i.e. blood, lungs and intestinal tissues which could be used for food grade protein products (Swingler).

The plasma fraction of blood is being increasingly valued as a meat substitute in comminuted meat products. In another method plasma protein for use in human nutrition is prepared by spinning of the plasma proteins. Plasma fibers contain 16-20% protein and are comparable to raw meat fibers. The concept of spinning is also being applied to proteins extracted from lung and stomach tissues. The NPU of spun products ranges from 53 for plasma to 77 for rumen fibers (Swingler).

Recently, solvent extraction processes have been applied for the preparation of fish (FPC) and meat (MPC) protein concentrates. Ethylene dichloride is employed as the extractant and the proteinaceous residue may be further treated with isopropanol to remove traces of fat and undesirable flavours in the products (Grant, 1976). FPC is produced from headed and eviscerated fish flesh (Hansen). MPC is prepared from offal tissues such as liver, lung, spleen, heart, kidney, stomach, blood and some bone (Grant, 1976). FPC or MPC preparations are expensive and the quality of the products has rather lower functional and nutritional quality than native fish or meat protein. Therefore its production has not been developed. The greatest needs for low quality FPC (drum dried from catches of small whole fish) are foreseen in certain South-East Asian countries with large populations of traditional fish consumers (Grant 1976).

Cheese or casein whey can be dried to whey powder. The uses of this powder includes baked goods, ice cream, processed meat and processed cheese. Deionised whey powder finds use in the production of so-called humanized baby foods. The Gel filtration, ion exchange or ultrafiltration aimed at extracting the protein from whey gives products suitable for use in the flour and sugar confectionary industries, in the soft drinks industry and in the preparation of baby foods of excellent nutritional value (Coton, 1976).

Unfortunately the high cost of raw material transportation, investment in sophisticated technologies, and high hygienic requirements, reduce the interest in whey protein production. Therefore the simplest means for whey utilisation is still to feed it directly to pigs.

4. Conclusions

The development of the use in food processing of non-conventional protein products obtained not only from soybeans but also from other raw materials will be promoted by the general world economy and changes in food availability. The development of new technologies can alter the specific functional properties and competitive position of the protein product obtained from different sources.

The improved nutritional profile and greater consumptional attractiveness of products will also influence the success of both the existing and future types of the non-conventional proteins as food ingredients. There are two principal conditions for good food: enjoyment eating it, and enjoyment of health as a result of food intake. Both are inseparable.

Actually we are at a stage when, in developed countries, vegetable protein products are considered to be substitutes, similarly to margarine 80 years ago. Technological advances in production and economic factors which may mean increased prices for animal products will soon account for a situation when NCP products will be regarded as regular elements of our food just as margarine is regarded now along with butter.

Legal, social and economic aspects - rather than technological ones - are the important constraints for the development of NCP as food grade products. They must be taken into account in the appraisal and calculation of any programme based on such products.

There is a strong tendency to conservatism in food habits. It is therefore reasonable to suppose that, as far as practicable, non-conventional food proteins (first of all SCP and BMP) will be used for the production of familiar food items, and feeding to animals for meat, egg and milk production will no doubt also be a common application in developed countries (Plaskett, 1976).

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THE MERITS OF EXTRACTED LEAF PROTEIN

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Potential abundance is the principle merit of protein extracted from leaves (LP). A paper for the IIASA conference in 1981 (Pirie, 1982a) put most emphasis on protein extracted from by-product leaves which would otherwise be wasted. There is little conflict of interest when such material is exploited. Provided the extraction can be managed economically, any such process is obviously advantageous. When crops are grown primarily as sources of LP, the case for diverting some land from conventional systems of agricultural needs more detailed attention.

In the U.K. the annual yield of dry, extracted, 100% protein can be 2 t ha^{-1}; in India, with no winter cessation of growth, the yield can be 5 t. It may be uneconomic to aim at such large yields because of the amount of fertiliser needed to attain them - practical yields will probably be 1.5 t in U.K. and 3 in India. Nevertheless, such yields are 2 or 3 times the yield of protein from any other source, e.g. a legume seed.

Fractionation by the method outlined here produces LP, the fibrous residue of the leaf, and a fluid containing various soluble leaf components. The dry matter (DM) of the original crop is distributed between these three fractions in the approximate ratios of 1 to 5 to 1. It could therefore be argued that the fibre is the main porduct and the LP a by-product. This is probably the situation in wealthy countries where there is no shortage of protein in the human diet, and where the number of cattle that can be kept depends on the supply of conserved winter fodder. More than half the protein can be extracted from a young, lush crop. Because various soluble components of the leaf are also extracted, the protein content of the residue from which LP has been extracted is not halved. The residue usually contains 1.5 to 2.0% N (on the DM); it is therefore a better ruminant feed than the best hay, but not as good as most of the "dried grass" produced commercially. Because juice has been pressed out of it, it is friable and can be dried by blowing air through it in summer even in Britain. When fuel is used for drying, the saving is considerable. During the past 40 years, this point has repeatedly been stressed: it is only now getting adequate recognition. An example will illustrate the scale of the potential saving. If pressing has been managed so that the fibre contains 65% water, 1.6 t of water have to be evaporated to get 1 t of "dried grass" containing 10% water. A good quality crop seldom contains less than 85% water and may contain more than 90% if harvested early in the day, or in wet weather, so as to keep the drying equipment in continuous use. In these circumstances, the weights of water that have to be

evaporated are 5.1 t and more than 8 t.

Cattle eat the product readily and several trials (mainly in the Rowett Research Institute, Aberdeen, but also in the USA) show that it has better feeding value than a crop with the same N content initially. This is because more of the N is true protein and the fibre, being from a less mature crop, is less lignified. Obviously, it contains less protein than the original crops, but a crop such as grass or lucerne, fertilised and harvested so as to give maximum yield, contains more protein than a ruminant needs. It is therefore reasonable to extract the excess for use by people and other non-ruminants. During the extraction of LP, much of the soluble material is removed from the fibrous residue so that it contains less strongly flavoured or toxic material than the original crop. Residues from plants such as water hyacinth and potato, which animals are unwilling to eat in the fresh state, should therefore be acceptable. This is a point that has still to be established by experiment. Nevertheless, one merit of LP production is that it could increase the supply of cattle fodder.

The effluent from silage is a troublesome pollutant that kills plants near the silo and fouls streams. There is no effluent from a silo filled with fibrous residue moistened with the fluid that is pressed from coagulated LP, so as to prevent access of air. Where conserved winter feed is important, LP production has therefore an environmental merit. The fluid contains most of the leaf K, much of the P, N in the form of amino acids and amides, and sugars. Ultimately, when there is regular commercial production of LP, it will be used as a culture medium for microorganisms. Several papers on its merits as a substrate have already appeared. It is not likely to be feasible to use the fluid in this way when LP is made on a domestic or village scale. It should then be used as fertiliser on an area of land similar to that from which the crop was taken. Silage effluent is toxic only because it is too concentrated locally.

With any crop, the annual yield of useful product depends in part on the area and duration of photosynthetically active leaf, and on the fraction of the total material made by photosynthesis that is finally present in the useful part of the plant. It is well-known that forage crops give larger yields than seed crops because, if harvested skillfully, land covered by them is continuously active photosynthetically with no period during which, as with seed crops, sunlight merely ripens and dries material that has already been made. Furthermore, all the above-ground material is useful. When human food is being made, these advantages of forage compared to seed crops are then lost because the forage is fed to an animal that returns in edible form only 10 to 25% of what was eaten.

There is an optimal time for harvesting a forage crop because, although the amounts of above-ground protein and dry matter increase steadily until maturity, the fraction of the protein that can be readily extracted diminishes as leaves age. Some examples of this are given elsewhere (Pirie, 1978); the reasons for it have been studied in some detail (Butler, 1982). Much research is still needed to find species and varieties that do not have to be harvested with uneconomic frequency in order to avoid the harmful effect of leaf maturity. As a result of that research, annual yields will probably increase. It is already possible to get a greater yield of edible protein from LP production than from any other form of husbandry; nevertheless, a technique in its infancy is being compared with techniques that have already benefitted from a vast amount of research.

Most of the LP used in human feeding trials is made from lucerne *(Medicago sativa)* because it is readily available. This increases the significance of the results because lucerne LP carries more residual flavour than LP from the cereals or from other legumes such as berseem *(Trifolium alexandrinum)* or

cowpea *(Vigna unuiculata)*. When unfavourable comments on the flavour of LP are made, it should be emphasised that they usually apply to material made from lucerne by people with questionable skill. There has been little work on extraction from bush and tree leaves. I have discussed elsewhere (Pirie, 1982b) the manner in which LP production could be integrated with "energy plantations". A perennial crop has obvious advantages in regions liable to heavy intermittent rainfall.

LP that will be used as human food should be made from crops harvested by mowing with an old-fashioned unit that collects the leaf on a belt without allowing it to fall on the ground. The leaf should then be washed to remove most of the surface dust: otherwise this will contaminate the LP. Any form of flail harvester is likely to introduce an unacceptable amount of dust into the product. This requirement may complicate very large-scale production of human food. However, one of the merits of LP is that it is well-adapted to small-scale production, and small-scale production will probably have a greater effect on the food supply of those now most in need than an increased supply of industrially produced food would have. Several engineering institutes and companies are working on the design of large-scale equipment for extracting juice from leaves. There is relatively little work on equipment for making a few kg of LP daily. The unit that we (Butler & Pirie, 1981) described is being improved at Rothamsted and by Dr. Joshi in the Department of Botany in the University of Aurangabad. A still smaller unit for family use is needed.

As soon as possible after juice has been extracted, it should be curdled by heating it quickly. Coagulation is complete in a few seconds at 70^0C. Heating to a higher temperature is advantageous as a means of decreasing the bacterial load that is inevitably carried by any agricultural product. Various proposals for other methods of coagulation have been made because 2 or 3 times as much energy is needed for heating as was needed for extracting the juice. It should however be borne in mind that material coagulated in any other manner would have to be heated later so as to ensure sterility, that with a little ingenuity in the use of counter-current flow systems much of the heat can be recovered, and that sudden heating prevents loss of LP as a result of proteolysis, and the formation of harmful substances such as pheophorbide as a result of other enzyme actions. All these points are discussed in detail elsewhere (e.g. Pirie, 1978).

Properly coagulated LP can easily be pressed to a cake containing 50% dry matter: with care the dry matter can be increased to 60 to 70%. Material as dry as that will keep for several days at tropical temperatures; for more prolonged storage, shelf-life can be increased by mixing it with salt, acetic acid or other pickling agents (Pirie, 1980). LP should be used in the moist state whenever possible because there is likely to be nutritionally harmful cross-bonding when a food containing lipids and carbohydrates as well as protein is dried. However, it is more convenient to make up diets of carefully controlled composition with dry material; that was used in all published feeding trials. Because of drying, the results of these feeding trials may under-state the merits of LP.

Skillfully made, dry LP contains 9 to 11% N, almost all of it in the form of true protein. Differences in amino acid composition between species are small (Byers, 1971) and there is no evidence that species differences (if any) are greater than differences between preparations from the same species harvested at different ages or after different systems of husbandry. Similarly, species differences have been claimed in the composition of the 20 to 25% of lipid that is present in LP from different species. About half the lipid is doubly or trebly unsaturated and it is well known that age and climate affect the degree of unsaturation of leaf lipids. Unsaturated fatty acids are essential components of the

diet. β carotene (provitamin A) is an even more important component of the lipid fraction of LP because vitamin A deficiency is widespread in tropical countries. Freshly made LP contains 1 to 2 mg β carotene g^{-1}, it is stable if protected from light and air, but is destroyed in the presence of air at rates that depend on the species from which the LP was made and the agents used for preservation (Pirie, 1982c). Because of the value of lipids as a source of energy, of unsaturated acids as essential fatty acids, and of β carotene for preventing the 200,000 to 300,000 new cases of blinding malnutrition that now occur annually, no attempt should be made to decolourise LP. It is inconceivable that this could be managed without loss of all these valuable components. People with a euro-american training in food science regard the dark green of chlorophyll, and its breakdown products, in LP as a demerit: such colours are not so regarded in most of the rest of the world. Fortunately, the techniques suggested for decolourisation are beyond the technical capacity of those to whom LP will be of most use.

Few communities eat dark green leafy vegetables (DGLV) to the extent that is both desirable and physiologically possible. It could be argued therefore that effort expended on making and popularising LP would be better expended on promoting DGLV. It is almost always a mistake to formulate such simple contrasts: each activity supports the other. Furthermore, the large annual yields of edible protein and β carotene attainable from DGLV have been publicised more vigorously by some of those concerned with work on LP (e.g. Pirie, 1976; 1981 and elsewhere) than by those whose primary concern is horticulture. LP and DGLV are allies, not competitors. Nevertheless, LP has the merit that it is better suited to infant nutrition than DGLV because of its small bulk. It is easy to supplement an infant's normal diet with 10 g of protein in the form of LP: it would be nearly impossible to give a supplement as large as that in the form of DGLV.

After experiments with chickens, fish, mice, pigs and rats had demonstrated that carefully made LP had the nutritive value expected from its amino acid composition, human feeding trials were started. Nitrogen retention by infants was nearly as good as when half the protein in their diets was LP and half milk, as when all of it was milk. Boys on a diet supplemented with LP grew more than those getting the same amount of extra protein (10 g d^{-1}) in the form of sesame flour. The signs of kwashiorkor disappeared in a few weeks when infants were given 10 g of LP daily by their mothers as a supplement to the home diet. That last trial was in Nigeria and the authors commented particularly on the improved demeanor of the children within a few weeks of getting the LP supplement. All these trials have been fully published (references in Pirie, 1978). In a more elaborate trial in Pakistan (Shah et al., 1981), 100 children, 7 to 14 years old, after getting any necessary medical treatment, were split into three matched groups and observed for 8 months while eating their normal unsupplemented diet, that diet supplemented with 200 ml of milk, or supplemented with LP made from mixed grasses and berseem. The LP supplied the same amount of N (1.4 g) and energy as the milk. The average increases in weight of the members of the three groups were 1.1, 2.45 and 2.6 kg, and in height 26, 48 and 53 mm. At the start, all had less than 12 g of hemoglobin in 100 ml of blood, at the end they had 11.7, 12.6 and 12.5. Clearly, in these circumstances, LP is as good a supplement as milk: it may even be a better one. It is possible that this unexpected result is the consequence of the β carotene in LP supplying vitamin A to a marginally deficient group of children. The LP was incorporated in 10 different dishes; an adult tasting panel judged all acceptable in appearance, texture, taste and flavour: some were judged excellent. That result is similar to our experience in Rothamsted where, if reasonable skill is used in presentation,

we find no problems with acceptance.

Only fragmentary accounts (e.g. Pirie, 1978; Devadas, 1981) of a similar trial have so far been published because it still continues. In each of 4 villages near Coimbatore (India) the midday school meal for about 60 children, 2 to 5 years old, is supplemented with 1.3 MJ, including 10 g of protein from different sources. In another village the meal is supplemented with tapioca to supply the same amount of energy but little protein, and a sixth village gets no supplements. All six get the same medical and educational attention. Because changing populations of children, rather than cohorts, are being studied, the experiment is difficult to score; it is however clear that the energy supplement makes little difference to growth or health, that milk is the best of the protein supplements, and that LP (mainly from lucerne) is as good as or even better than protein in legume seeds. Other similar trials have been started in other parts of the world.

In all these trials there was some coercion, or the food containing LP were supplied as part of a "package" which contained some desirable components. So, although those responsible for the trials in Pakistan and Coimbatore comment that within a few months the children given the LP supplement came as regularly and willingly as those given the other supplements, these trials do not demonstrate that LP would be eaten if no inducement were coupled with it. When adult prejudices do not interfere, there is no difficulty in giving LP to children just after weaning; their diet is changing in any event and they have not acquired any prejudices. But LP is not as digestible as milk; milk, when the supply is limited, should therefore be reserved for the youngest children. It is the older ones and nursing mothers, who are more effectively equipped with digestive proteases, who should get the LP.

Results such as these should soon dispel the initial reservations that most scientists had about the value and practicality of LP as a dietary component. That is a first small step towards winning acceptance by the public: people, as a rule, eat what is traditional until their food habits are altered by emulation or advertising pressure. The common assertion that food habits are hard to change is false: indeed the readiness with which they change is partly responsible for the present food problem. Communities that used to eat locally grown yams are now demanding wheat, and mothers who used to suckle their babies are buying artificial foods for them to an increasing extent. I give more examples and discuss the reasons for change, and the problems arising from it, elsewhere (Pirie, 1972; 1981; 1982d).

The potential abundance of LP is the main reason for confidence that it will be used in some form. Though the basic principles of production will remain the same, differing styles of production are appropriate in an isolated village and a large commercial farm; or in the humid tropics and a region with a prolonged winter. Research is therefore needed in several environments, with several objectives, with many existing species and varieties of leaf, and with new varieties selected for prolonged vegetative growth. There will be little incentive to undertake such research for as long as there is uncertainty about the merits of the products and the economic prospects of the process. Quality should therefore be judged first of all on fresh material made with more care than it might be possible to deploy in actual practice: the harmful effects (if any) of simplifications should then be studied. The costs of growing and collecting crops are known from other aspects of agriculture; it is the cost of power, labour and equipment for processing that are uncertain. Any process can be made to seem uneconomic if a sufficiently unsuitable system is used to operate it. In principle, the amount of energy that has to be expended in liberating and expressing leaf

juice is small: so is the pressure needed for extraction. The problem is to find means for keeping these factors small in practice.

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PROSPECTS FOR FOOD PROTEIN PRODUCTION FROM NON-CONVENTIONAL SOURCES IN THE CZECHOSLOVAK SOCIALIST REPUBLIC

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1. Introduction

The present and future protein source situation throughout the world is among the most serious problems in human nutrition as well as in the nutrition of animals. In Czechoslovakia, the consumption of food has shown a gradual transition from plant sources to animal sources; this is manifested mainly in an increase in the consumption of meat and meat products, milk and eggs. Over the past 20 years (since 1960), the consumption of meat and meat products, expressed in the values of meat-on-bone, has increased from 56.8 kg/person/year up to 85.6 kg/person/year. A similar increase is recorded in the consumption of milk (in values of liquid milk) from 173 litres to 233 litres, and in the consumption of eggs from 179 to 316 eggs per person annually.

This consumption trend also manifests itself in an increased consumption of protein per average inhabitant. The daily total protein consumption has increased from 86 g to values close to 100 g (96.8 g in 1980). A marked increase is recorded mainly in animal proteins where an increase from 41.2 g to values about 57 g has been obtained. This is to say that in 1980 the total consumption of protein was provided by animal protein at a rate of almost 59%. The high trend of the consumption of animal protein has also another manifestation; the consumption of lipids has markedly increased together with protein consumption which is, however, an undesired phenomenon. The consumption of lipids has increased from 102.5 g/person/day to as much as 117.7 g/person/day which exceeds the recommended food allowance level by more than 23%.

Besides the adverse health effects of a higher consumption of animal proteins (in raw material always combined with a larger amount of lipids), the increasing demand for food of animal origin also has an adverse economic impact. The production of animal protein is fairly costly, as compared with vegetable protein and it is 10 - 14 times more laborious. By Vigner's calculation, 1g of animal protein requires an average input of 8.2g of feed protein. Under Czechoslovak conditions, the losses and reserves in production, processing, marketing and consumption amount to as much as 30 % of the produced amount of protein.

All these facts call for a maximum effectiveness of the management of animal protein production, for its use at the highest possible rate directly for human nutrition, for reducing its loss throughout the food chain, and for seeking non-traditional sources of raw materials to replace, at least partly, the animal

protein sources. Such a raw material may be seen in the concentrates of non-traditional protein sources which are not used at present in the Czechoslovak food industry, or are only used to a minimum extent.

Owing to the fact that the production of slaughter animals is among the most expensive farming activities, the use of non-traditional protein sources will be effective everywhere in replacing costly raw materials. First of all, replacers can be used as additives to crushed meat products and in the commercial production of ready-made foods. Naturally, protein concentrates may also be used in other fields of food production where they favourably influence the technological properties, improve the organoleptical characteristics and nutritive value, increase the use value of food products (higher durability, wider spectrum of use, time savings in final treatment), and enable wider innovation of new products. However, replacement of part of meat raw material is the main use for non-traditional protein.

Non-traditional protein concentrates must meet the basic technological requirements in order to be able to replace part of the meat raw material. These requirements are water-holding capacity, emulsifiability, good organoleptical properties, good hygienic characteristics, and reasonable price. If all these requirements are met, non-traditional protein concentrates can be applied with success.

What is the expected future demand for protein concentrates in Czechoslovakia? The possibility of using protein concentrates derives from the output of meat products and commercially produced ready-made meals, and from the proportions of concentrates in these products.

In the food industry, the annual output of small and soft meat products, i.e. the best foods to which non-traditional protein can be added, is 200 to 250 thousand tons. The currently permitted 3% supplement on non-meat protein, saving the organoleptical value and technological processing quality, makes it possible annually to apply 6000 to 7500 tons of protein concentrates to these products. Boiled products, cans of luncheon meats and pies represent another possible field of the application of protein concentrates in food industry; however, its importance is lower, as to the volume of output.

However, non-traditional protein concentrates can find a much wider field of application in the development of the commercial production of foods for public catering. From the technological point of view of the production of ready-made foods, it will be necessary mainly to provide homogeneous raw material: this is possible by the technology of reconstituted meat using the REMA of COMMITROL systems. As Kusiak's data indicate, a 10% proportion of commercially produced ready-made meals in factory and school catering will represent 108 thousand tons of these foods in 1985. This is the minimum variant; the optimum proportion of ready-made would be 20%. The minimum proportion of commercially produced meals for the year 1990 is expected to be 25%, i.e. 350 thousand tons, whereas 50% (700 thousand tons) would be the optimum - but unrealistic - level. The meat component of these meals is about 10%; if 15% of non-traditional proteins is assumed to be applied in the form of concentrates (which is realistic from the point of view of technology and organoleptical characteristics), the requirement for non- traditional protein concentrates for these purposes would be 1500 tons already in 1985; in 1990 this amount would increase to 10,000 tons of concentrates at a minimum if the optimum concentrate level is to be provided. It should be stressed again that the optimum means only 50% of factory and school catering and that restaurants and other types of catering (hospitals, army and the like) are not taken into account - in these cases the demand for concentrates would certainly be

even higher.

This consideration does not take into account the possibilities of introducing in the market new innovated products which might partly replace traditional meat products in consumption. These new products would naturally be different in their properties and uses from the traditional products; as such, they would need much publicity at the beginning in order to change the existing food habits and to develop new ones. The consumption of concentrates for this field is hard to estimate since such products are not manufactured at present and even the experience from abroad is just sporadic or at the research level.

Now let us consider the availability of raw materials for the production of protein concentrates in Czechoslovakia. It should be borne in mind, first of all, that the climatic conditions of the CSSR do not allow for a wider use of soybeans as the most widespread and best-elaborated source of vegetable protein. Soybeans are just a marginal crop in Czechoslovakia; only part of the groats from the production of soybean oil has some importance for the production of food protein concentrates for which a project is now in preparation which, if accepted, will be in operation by 1985 (soybean protein concentrate, extruded soybean flour). Owing to the geographical positon of Czechoslovakia it is also impossible to use the proteins from sea products, mainly krill.

The widest possibilities of using vegetable protein lie in the newly developed and tested technology of the separation of fodder wheat flour in the form of suspended starch and emulsified proteins. The final product is wheat protein concentrate containing 35% of proteins, suitable for meat products as a replacer for the currently used milk powder; it has good functional properties (supplement of up to 3%) and is able to replace up to a two-fold amount of the corresponding quantity of the meat used for production. Its drawback is a limited spectrum of use, owing to a low biological value. This problem can be solved by combining it with an addition of lysine obtained by fermentation. Other products of this technology include starch and its products (food starch and - after hydrolysis - also fodder starch) in which an appropriate technological treatment also suitably increases feeding value.

Extracted rapeseed meals are another important source of vegetable protein; rape is the most widespread oil-bearing crop in Czechoslovakia. What makes its use for human consumption difficult is the high content of antinutritive substances whose removal is still very laborious and costly. Breeding may help to solve this problem: the requirement for a reduction of the content of undesired glucosinolates is not unrealistic but for the time being it appears to be a matter of future efforts. Another requirement is to retain the currently obtained yields of rapeseed.

Potato protein is the best from the biological point of view. However, the raw material contains a very low amount of protein (2%) which is therefore hard to obtain. The efforts to utilize it have always failed due to the need for removing much water and to the low protein retention. The protein of commercially processed potatoes cannot be used for human consumption unless th- starch industry is subjected to costly reconstruction and unless new technologies of potato starch are introduced (which is a lengthy and costly process).

Another source of biologically full-value protein is the protein of skim milk. Methods of the production of caseinates, coprecipitates and ultrafiltration concentrates have been worked out for food-production purposes. About 36 000 tons of milk protein was fed to animals in 1980. If 10% of this amount were used as sodium caseinate, about 3300 tons of food concentrate would be obtained and a great quantity of whey would remain for feeding purposes. However, the high energy requirement, mainly for whey drying, has arrested the efforts for making

the project reality, for the time being.

The use of blood protein is a reserve inherent in the meat industry itself. About 2.2% of extracted blood protein is being used for human nutrition at present, the total potential of blood protein being about 10,000 tons. For the time being, wider use of blood is hindered by a lack of equipment for commercial blood collection and by the bad organoleptical properties of untreated blood. A solution can be seen in the introduction of imported equipment for the collection and treatment of blood based on plasma separation. This would secure the utilization of about a third of the protein potential, used in a sensorically (organoleptically) acceptable form. Another possibility is to use emulsified food paste consisting of blood, casein and fat. A larger proportion of blood is still to be fed to animals.

The use of the proteins of monocellular organisms appears to be most promising from the point of view of commercfal production. This method of production has the following advantages: quick and industrially well-organized production, a large proportion of protein in dry matter, the use of waste as fermentation raw material, possibility of purpose-oriented selection of suitable strains, independence of environment, and high labour productivity. Health experts claim that yeast cultures are the best for this purpose: there is already some habit of their use (in many cases they are natural part of food) and, in comparison with bacterial cultures, there is a lower hazard of undesired biochemical processes and thereby a lower risk of the rise of abiogenic compounds and of the production of toxins.

A new technology of yeast protein production for human nutrition has been developed in Czechoslovakia; it is based on a complete utilization of the yeast cell. Besides the production of yeast protein concentrate for the meat industry, this technology enables the production of biochemicals (NAD, NADP, analytic enzymes, zymosan), drugs (ergosterol, glutathione, RNA), diagnostic media, food appetizers, growth stimulants, lipid fractions for cosmetics and other products. Protein concentrate contains 80% of proteins and maximally 1% of nucleic acids. The problem of suitable and available substrates was solved at the same time. The use of magnesium sulphite extracts is ready for practical introduction and the use of available lignocellulose materials (straw, wood waste) is being considered. Research is required for securing continuous production and for reducing the energy requirement for these purposes. Another substrate still not utilized to full effect is milk whey; the procedures of its fermentation for binding inorganic nitrogen (ruminant feed), for the production of yeast protein, for the use of lactose and the like are known.

Within the complex approach to the food program in Czechoslovakia, studies are being conducted concerning the most efficient utilization of all available reserves for the production of food protein concentrates, aiming at reducing the national-economic pressure upon animal production and at introducing new processes in the food industry with an increased utilization of available raw materials and with a reduction of losses. However, the practical introduction of such processes depends on the capital available to the food industry in the near future.

THE ROLE OF PROTEINS PRODUCED BY NON-CONVENTIONAL TECHNOLOGIES IN NUTRITION OF MAN AND HIS DOMESTIC ANIMALS

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Introduction

The basis of mammals nutrition lies in the supply of protein and energy according to their nutrient requirements. The bio-synthesis of proteins is the *sine qua non* of the growth processes in the sense that growth increments to the body tissues always consist largely of protein (Mitchell 1962). Therefore, protein nutrition of man and simple stomached animals highly depends on the biological value of protein owing to their insufficiency to synthetise essential amino acids in their body in appropriate quantity. For food to be used with maximum efficiency they must receive the essential amino acids in the correct quantities and relation. Only the ruminants are almost independent of quality of dietary protein thanks to microbial activity in the forestomachs.

Beside the intensification of plant production and increasing the yield of animal protein through the more effective conversion of photosynthetic products in animal husbandry, the utilization of non-conventional technologies for protein production is the other way. Because of the increasing scarcity of some crucial resources for agricultural production (e.g., land, energy) on the one hand and the need to secure the nutrition for growing human population on the other hand the conversion of all available raw materials to food, or feed is desirable.

1. World Demand for Higher Agricultural Output

The major nutritional problem much of the developing world faces today is the growing imbalance between the rate of population increase on the one hand and the rate of increase in food production on the other. In developed countries agricultural technology is much further advanced but, because of other limitations, food production has grown at the same average rate of 2.7% per annum as in the developing countries. However, since population growth is much lower in the developed countries, food production per head has risen (FAO 1969).

It is quite likely that the apparent association between economic wealth and a high level of agricultural output is important. The increases in wealth are associated with an increase in the demand for milk and meat. The wealth is also associated with a considerable degree of industrialization (Blaxter, 1970). The conditions for sustained economic growth usually depend on a high level of agricultural productivity and transfer of labor to industry (Rostow, 1960). The food problems mainly tend to arise in the low income countries where agricultural

technology is at low level (Bunting, 1970).

According to the FAO, 800 million people suffer from shortage of food. In spite of the fact that 70% of the world's inhabitants are living in these countries, only 49% of the world's cereals are harvested there. There is a great difference in calorie intake in different countries as may be seen from FAO (1979) data which appear in Table 1. A great contradiction follows from protein consumption (Table 2). Especially, consumption of animal protein is substantially higher in developed countries than in developing ones The lack of protein of high quality, especially, leads to malnutrition and undernutrition in developing countries. According to FAO statistics at least half mankind suffers from protein deficiency, mostly owing to the lower animal protein consumption and to the inadequate composition of protein found in cereals. The situation is most difficult with children, who need exceptionally large amounts of essential amino acids for the growth of tissues. According to the FAO-WHO (1965) report, children 10-12 years of age need approximately 0.7-0.8g protein, adult only 0.5-0.6g protein per kg of body weight per day. Damage caused by the lack of protein during childhood cannot be cured later, at least not completely. Protein malnutrition results in kwashiorkor and high infant mortality. Ignorance, apathy and, in general, the lack of activity contribute to the nutritional difficulties in the adult. People suffering from the lack of protein are thus in a witch's cage from which it is difficult to escape (Virtanen, 1968).

Table 1. Calorie intake per capita per day in 1975-1977 (FAO 1979)

Country	Total	Vegetable Products	Animal Products
World	2,590	2,149	441
Africa	2,307	2,140	167
N.C. America	3,215	2,195	1020
U.S.A.	3,537	2,237	1,300
S. America	2,565	2,077	488
Asia	2,277	2,077	200
Europe	3,410	2,315	1,095
Oceania	3,203	2,026	1,177
Developed all	3,373	2,336	1,037
Developing all	2,282	2,075	207

Many plans for the removal of the protein deficiency of undernourished people have been made during the last few years. Great attention has been paid to entirely new methods for protein production. New ways for protein production are promising under existing conditions of demographic explosion because traditional methods of obtaining feed and food protein are limited by inadequate energy sources. Natural ecosystems are characterized by essentially complete recycling of energy and nutrients. Offtake of plant products usable by humans or animals usually is in proportion to the input of energy in the form of human or animal labor, fuel, fertilizer, pesticides, etc. Thus additional energy is supplied to increase the conversion of solar energy to chemical energy in primary plant production. Part of the human population which depends only on renewable energy cannot afford a substantial portion of human food. The protein poor part of the world which does not use much fossil fuel consumes 9 grams of animal protein per capita per day as against the world average of 44 grams. Additional small inputs of coal and petroleum compared to the solar energy are

Table 2. Protein intake per capita per day in 1975-1977 (FAO 1979)

Country	Total (g)	Vegetable Products (g)	Animal Products (g)
World	69.2	44.8	24.4
Africa	58.7	46.7	12.0
N.C. America	92.7	36.2	56.5
U.S.A.	106.2	33.5	72.7
S. America	66.1	36.8	29.3
Asia	58.3	46.2	12.1
Europa	96.0	43.2	52.8
Oceania	95.8	33.3	62.5
Developed all	98.4	43.3	55.1
Developing all	57.8	45.4	12.4

significant and have explained the difference in our way of living (Altschul, 1966).

Animal protein is enjoyed mainly by 400-500 million of the planet's 3 billion inhabitants. Cereals are the major source of protein for humans. They contribute over 40 million tons of protein annually to the world's human diet as compared to 25 million tons from animals and 12 million tons from legumes and nuts (Asltschul, 1966).

In future either increasing the efficiency of the products of photosynthesis and introducing non-conventional technologies for protein production from raw materials of agricultural or non-agricultural origin methods must be taken into consideration to increase protein production.

2. The Contribution of Non-conventional Technologies for Improving Protein Nutrition of the Human Population

Wide-spread cereal proteins are deficient in several amino acids. Biological value is increased after supplying the other protein, especially of animal origin, or by synthetic amino acids.

There is a world shortage in production of animal proteins which are superior in nutritional quality to plant proteins. Animal production can be expanded neither easily nor rapidly to overcome the deficit. The magnitude of the imbalance between production and need for animal protein will become worse as world population increases. The FAO (1964), suggested that by the year 2000 a threefold increase in total protein supplies and a fivefold increase in animal protein supplies would be needed in the developing countries.

Table 3 shows that energy and protein conversion to foods for man are most efficient via milk and eggs production and lowest in lamb and beef production. However, in term of protein production per unit of metabolisable energy, poultry slightly exceeds milk production and the pig occupies an intermediate position. An increase in crop production is undoubtedly the quickest way of improving world food production. Estimates by Wilcke (1966), indicate that an acre of land will provide a human's protein requirement for only 77 days in form of beef, 236 days as milk, 773 days as corn meal and 2,224 days as soya beans.

Table 3. Efficiency of conversion feed nutrient to edible product (Wedin et al. 1975).

Livestock	Crude Protein %	Energy %	Gross Edible Products as % Feed Intake
Broiler	23	11	45
Hens (eggs)	26	18	33
Swine	14	14	30
Dairy cattle	25	17	90
Beef	4	3	10
Lambs	3	-	7

The degree of direct competition between animals and humans for food depends, to a certain extent, on the type of livestock production. Beef and milk cows fed high roughage diets compete less directly with humans for food, as these animals can convert forages and other feed by products inedible to humans, into the high quality animal products most desired by humans.

Table 4. Annual yields from animals and from crops (Holmes 1970)

	Energy (Mcal/ha)	Protein (kg/ha)
Dairy cows	2,500	115
Dairy and beef cattle	2,400	102
Beef cattle	750	27
Sheep	500	23
Pigs	1,900	50
Broilers	1,100	92
Eggs	1,150	88
Wheat	14,000	350
Potatoes	24,000	420

2.1. Amino Acids Supplementation of Cereals

For at least 5,000 years cereals have predominated as the arable crops grown to provide food for man and animals. The importance of cereals in the nutrition of people is increasing. Caloric yield per unit of arable land is greater for cereals than for other crops. Their protein content is too low to sustain optimum growth in simple stomached animals and man. The cereals are being improved as protein sources by supplementation with animal and protein concentrate. Similarly supplementation of cereals with pure amino acids results in an increase of the amount of utilizable protein. Protein needs of children or adults may be supplied by the fortified cereals alone. Among the essential amino acids, L-lysine and methionine rank first in order of importance followed by threonine (rice) and tryptophan (corn). Cereals supplied in amounts adequate to cover caloric needs can be substantially upgraded with the mentioned amino acids. Industrial production of synthetized amino acids needed for supplementation of cereals has given a theoretical possibility to improve the nutritional value of flour and diet for pigs and poultry (Ottenheim and Jenneskens, 1970).

2.2. Genetic Improvement of Cereals

An improvement of economic status in developing nations, particularly in those in the tropical belt around the world, will lead to an improvement in their diets. The protein deficiency was much more frequent than the caloric deficiency. Supplies of animal foods are, however, too expensive for those who most need proteins. One approach is to change the protein of crops genetically to obtain a more balanced amino acid profile. Maize, deficient in lysine and tryptophan, is a staple food in many countries. Improvements of its protein quality could offer hope for millions of people. After analyzing hundreds of maize varieties in mutant types a strain of maize, Opaque-2 was found by Mertz et al. (1965) to be exceptionally high in lysine and later on in tryptophan, too. About 300 grams of this maize are needed to maintain protein status in the adult. Unfortunately, maize is rather a demanding plant which is not suitable for cultivation in wide areas of the globe. Sorghum and rice are subjects of similar research.

2.3. Newer Proteins for Man Nutrition

Oilseeds, particularly soybeans, cotton seeds and peanuts, are the major low-cost source of protein. Concentrates from oil-cake have good nutritional value. The usage of non-animal protein as an extender of animal protein is rapidly increasing. A complete diet for humans without animal products is readily attainable.

With an increased world wide demand for animal protein total conversion of muscle into meat must be most effective. The using of mechanical deboning is possibly increasing meat yield from a carcass. Levin (1979), described technology of a meat protein concentrate production from internal organs. Blood from slaughtered animals is a potential source of a high quality protein. Serum proteins are possible to be spray dried in the presence of lactose. Similarly whey is spray dried either alone or delactosed (Satterlee, 1975). Dry whey is a very desirable product for protein fortification, too.

Extraction of plant juice protein from green plants for concentration as a protein for man and simple stomached animals with residues for ruminants is an example of green plant fractionation. Koegel et al. (1974), indicate that on a ha. basis about 1,120 kg of protein concentrate may be produced from alfalfa.

The cultivation of unicellular green algae in an inorganic nutrient solution has been the object of very great attention. Although algae contains more than half of the dry matter as a protein, utilization of protein of a fresh mass is only 25%. After the cell walls are broken and after drying the utilization of the protein obtained markedly increases. Also the separation of algae from the nutrient solution is not easy. Probably a very cheap protein cannot be produced by this way (Virtanen, 1969).

The production of yeast protein is the other promising way under the condition that a cheap substrate for cultivation is available. Nucleic acid content is one of the greatest limitation of nutritive value. Thanks to high B-complex and protein content yeast has been used in human nutrition. Thus in the World War II about 15,000 tons of food yeast were used per year in Germany to supplement human foods (Smith and Palmer, 1976).

3. Protein Feeds Produced by Non-Conventional Technologies

The efficiency of the conversion of plant origin feeds to animal protein depends mainly on the protein availability and balanced content of essential amino acids. The unbalanced content of essential amino acids leads to lower

animal productivity and decreases livestock commodity production. The efficiency of feed conversion can be improved if protein supplements are used in feeding of animals. There are some non-agricultural technologies for production of protein supplements. After Milner et al. (1978) these technologies are classified into photosynthetic and non-photosynthetic single-cell protein production, leaf protein production and the product of chemical synthesis. Comprehensively speaking agricultural waste and secondary products may be used either for microbiological synthesis or for direct use in feeding cattle. The practical exploitation of new technical processes is strictly bound to economics. The demand for bioproteins will be probably affected by the market supply of soybean meal and fish meal as well as by the total demand for protein rich feeds.

3.1. Single Cell Proteins

Bioproteins, also called single cell proteins, can be produced by cultivating bacteria, algae, fungi and yeast on simple energy containing substrates, e.g., hydrocarbons and carbohydrates. Based on chemicals (n-paraffins, methanol, etc.), different carbohydrates and industrial wastes (sulphite liquor), bioproteins may be potential carriers of toxic elements. Until their use for human consumption is better clarified, they have to contribute to the human food only indirectly as a feed for animal production (Hanssen, 1981).

The bioproteins are important primarily as protein supplements to cereals. The various kinds of bioproteins are characterized by a high protein content, relatively rich in lysine which makes them specially valuable in completing cereal protein. And moreover, the cereal protein supplements the bioproteins which are lower in methionine and cystine.

3.1.1. Algae

These are simple plants able to grow in a pure inorganic culture media and in suitable conditions they could produce considerable quantities of protein. Species of algae such as *Chlorella*, *Scenedesmus* and *Spirulina* can be commercially exploited and fed to pigs (Oswald and Golueke, 1968).

3.1.2. Yeast

For yeast production a great variety of microorganisms including *Torula*, *Candida* and *Saccharomycetes* have been investigated. Also a range of different carbohydrates has been used, cereals, sugars, molasses, waste sulphite liquor, cheese whey and even sewage. The amino acid composition of yeast, (Table 6), is generally good but relatively low amounts of sulphur amino acids have been noted (Braude 1976). Some of the early studies on utilization of n-paraffins or gas oil were successful. However, due to high oil prices and partly to possibility of some hygenic problems production has been limited. Good results were obtained in the case of the addition of synthetic ethanol to sulphite extracts. Charatjan and Wolnova (1975), received the best results with the yeast grown on medium containing ethanol as a sole carbon source. Similarly, the sulpnite-ethanol yeasts were found to have higher biological value of protein (69.4) than sulphite yeast (65.8) (Šimeček 1971).

For improving the biological value of bioproteins Smith and Palmer (1976), recommended the addition of 0.18-0.44% methionine. Microbial cells contain more non-protein nitrogen in the form of nucleic acid than most conventional sources do and RNA and DNA comprised about 10% nitrogen in hydrocarbon-grown yeast, whereas only 1% was found in fish meal.

3.1.3. Bacteria

Recently some petrochemicals (e.g., acetic acid, methanol, ethanol) have been used as a source of energy for cultivating bacteria. The continuous fermentation process involved in the production of Pruteen from methanol using strains of *Pseudomonas* was reported by MacLennon et al. (1973). The technologies developed by Imperial Chemical Industries Ltd. are using low concentration of methanol as an energy substrate and ammonia as the nitrogen source for the protein biosynthesis of the organism classified as *Methylophilus methylotropus*. Bacterial protein isolates may contain as much as 830 grams of crude protein. However, such a high value may be misleading, because about one quarter of it can consist of nucleic acids, which on present evidence, cannot be utilized by single stomached animals (Braude, 1976).

The other product Toprina is being produced by cultivation of *Candida lipolytica* on n-paraffins. Mitsubishi Protein Concentrate is being produced by cultivation of *Pichia aganobii* on methanol in Japan.

3.1.4. Fungi

A process has been developed recently in Finland which involves a continuous cultivation of the filamentious microfungus *Paecilomyces variati* on sulphite spent liquor. Preliminary results indicate that the product named Pekilo protein can satisfactory replace fish meal as a protein supplement in the diets of growing pigs. Products of this type may acquire special importance if one takes into account that wood is the only available renewable natural resource.

3.1.5. Nutritional Limitations of Using Single Cell Proteins

The nutrient content including several amino acids in case of Pruteen, Toprina, Pekilo and MPC are presented in Tables 5 and 6. The nitrogen fractions consist of true protein, nucleic acids and other non-protein nitrogen. Due to the fast growth of micro-organisms bioproteins have a high nucleic acid content, mainly RNA. Pruteen, Toprina, Pekilo contain nucleic acids expressed as percentage in crude protein 18.9%, 14.3%, and 18.1%, respectively (Hanssen, 1981). Nucleic acid content in feed for animals is of interest both from health and nutritional point of view. They consist of purine and pirimidine bases which are metabolised and broken down in the organisms. After absorption into the body purines are eventually catabolized to uric acid. In order to eliminate it the action of the enzyme urate oxidase is essential to convert it to allantoin which can be excreted in urine. It is known that primates lack urate oxidase and cannot effectively eliminate large amounts of uric acid (Braude et al., 1977). In addition in humans the uric acid has bee connected with the formation of renal stones and developed of hyperuricemic nephropathy. For humans it is therefore recommended to limit daily intake of nucleic acid originating from bioproteins to two grams (PAG 1975). In contrast to this allantoin is formed and excreted in animals, nucleic acids do not represent any health risk to them.

Table 5. Nutrient content in Pruteen, Toprina, Pekilo and MPC (Hanssen, 1981)

Bioprotein	Pruteen	Toprin	Pekilo	MPC
Dry matter %	90.4	95.1	90.5	94.2
Ash %	8.3	7.1	5.0	7.8
Crude protein %	68.8	53.8	40.8	53.5
Nucleids acids, % as feed	13.0	7.7	7.4	8.3
Nucleic acids, % of cride protein	18.9	14.3	18.1	15.5
Ether extract %	9.5	11.2	4.4	5.8
Crude fiber %	0.8	0.4	8.0	0.2
N-free extracts	3.0	22.6	33.0	27.5
Phosphorus g/kg	18	15	12	19
Calcium g/kg	0.5	0.2	3	0

Table 6. Amio acid composition (g amino acids/16 g N) and protein utilization in different bioproteins.

	Pruteen	Toprina	Pekilo	MPC	Soybean meal
Valine	5.89	5.35	4.81	5.19	4.79
Isleucine	4.99	4.62	4.10	4.96	4.65
Leucina	7.61	7.13	6.61	7.19	7.67
Threonine	4.77	5.04	4.11	4.50	3.96
Serine	3.55	5.43	4.33	4.56	5.23
Methionine	2.34	1.64	1.57	1.58	1.31
Phenylalanine	3.94	4.44	3.64	4.45	5.51
Tyrosine	3.46	3.53	3.17	4.04	3.74
Lysine	6.42	7.10	6.12	6.90	6.47
Cystine	0.68	1.18	1.09	1.48	2.02
Tryptophane	1.31	1.24	1.18	1.40	1.29
Arginine	5.38	4.79	6.0	4.95	7.69
Histidin	2.31	2.34	3.00	2.15	2.57
Chemical score	45	41	37	40	53
EAA-index	78	76	70	77	78
True digestibility	-	83.8	77.2	86.3	83.3
Biological value	-	74.6	77.0	86.1	80.5
Net protein utilization	-	62.5	59.4	74.3	67.1

Bioproteins can therefore be fed to pigs and other animals in fairly large amounts (Hanssen, Farstad, 1980). Although too much nucleic acids in the feed seems even to lead to a net loss of protein (Roth, Kirchgessner, 1980). The removal of nucleic acids from the yeast to receive depurinished dried yeast represents another way (Trevelyan, 1976).

In some experiments negative effect of more than 10-15% Toprina in feed was observed (Van der Wal, 1976). Similarly Pruteen seemed to have specific pathological effect in kidney in some cases. The presence of endotoxins was not excluded as a possible cause. In spite of this, in other experiments Hanssen and Farstad (1980), did not demonstrate any adverse effect using as much as 25% Pruteen in the ration of pigs.

The bioproteins as feed are characterized by a high lysine content and a comparatively low content of sulphur-containing amino acids (Table 6) which are the first limiting amino acids. Both Pekilo and Toprina showed inferior value for biological value of protein and net protein utilization as compared to soya protein. Apparent digestibility coefficients of the crude protein close to 90 for Toprina and Pruteen and 85 for Pekilo were found.

The cultivation medium has a greater effect on the nutritive quality of SCP than the strain of microbe. Perhaps the SCP from sulphite liquor has a little lower digestibility than the SCP from some other energy sources (Nehring et al., 1970).

Microbe products contain about 35% carbohydrates and 4-10% fat (Salo and Pekkarinen, 1981). The composition of both these groups differs greatly from that of conventional feeds. Hexoseamines are typical of the microbial products. Sugar content was very low and starch was absent. The digestion of yeast cell wall polysacharides, composed of glucan and manosan, by the digestive enzymes of the calf small intestine is very small. In feeding broilers and calves, some restriction has been introduced (Kossila and Kiskinen, 1978). Crude fiber content is low with exception of Pekilo which contains 10% of it. The calculated metabolizable energy content for Pruteen, Toprina and Pekilo is 14.4 MJ/kg, 15.8 MJ/kg, and 12 MJ/kg, respectively.

Bioproteins are rich in phosphorus but poor in calcium. Yeast and Pekilo are traditionally regarded as a good source of B-vitamins except for B_{12} vitamins.

3.2. Biological Role of Ruminants in Processing Raw Materials

At present ruminants are the major practical convertors of cellulose to animal product such as milk and meat. Thanks to microbial activity in a forestomach they are able to change cellulose and low quality protein to high palatable animal protein. Instead of cellulose, new carbohydrate sources such as hemicellulose non-utilizable by simple stomached animals and non-protein nitrogen may be used for animal protein production. Owing to utilization of roughage, by-products and waste there is not strong competition with human consumption of cereals. In addition ruminants can obtain food from areas not accessible to cultivation.

Hydrolytic reaction during decomposition of cellulolytic materials as a preliminary step for SCP production is a highly energy consuming process. The increasing prices of energy and higher cost of SCP production form presuppositions for utilization of ruminants as effective convertors of renewable resources to SCP in forestomachs and which create milk and meat after that. But for improving digestibility of lignocellulose materials previous technological treatments are recommended.

3.3. Lucerne and Grass Juice as a Feed

Mechanical extraction of juice from lucerne and grass is now commercially feasible. This technology seems to be attractive because it can not only provide a valuable source of protein for single stomached animals but also makes

considerably cheaper the preservation of these feeds for ruminants by drying. This way may increase home-produced proteins for pigs. Fibrous green crops can be separated by mechanical methods into two fractions - high quality fibrous feed for ruminants and a liquid rich in protein, sugars and minerals for non-ruminants. The yield of crude protein from grasses and legume crops is substantially greater than for other methods of crop or animal production (Houseman and Connell, 1976).

		Yield of protein /kg/ha/year
Animals	-intensively reared	100-200
	-daily herd	250-300
Crops	-wheat grain	550
	-field bean	840
Leaf protein	-lucerne	1,100

The pulp residue can be utilized by ruminants in the fresh, dried or ensiled form. The extracted juice can be utilized for non-ruminants feeding in the liquid form or as whole juice dried or as the liquid coagulated juice. The preparations of the whole juice dried or dried leaf protein concentrate have been made for use in diets for poultry and man. Amino acids are present in amounts comparable to high protein but methionine is the first limiting amino acid. The breakdown of leaf protein is prevented by heat treatment of the juice within 1 hour after production and by reduction of pH to approximately 3 (Braude 1976).

It appears that the liquid form represents the most attractive way of feeding lucerne juice to pigs. After Barber et al. (1980) the juice may supply half of protein supplements in the diet of pigs. The dried protein rich coagulum, known as LPC, is similar in nutritive value to the more conventional protein sources. This product produced in the USA is called Proxan. It is apparently well utilized by pigs but economy of production has been recently questioned.

3.4. Animal Wastes

Animal wastes should be recognized as an active biomass since they are the product of metabolism in the same manner as the by-products of the feed industry are which involve fermentation. The shortage of feed protein for animals results in the study of possibilities of animal wastes recycling as feedstuffs. Problem of disposing wastes was born with intensive animal production systems and high concentrations of animals.

3.4.1. Dehydrated Poultry Waste (DPW)

It includes high temperature dried waste which contains droppings and spoiled feed from cage layers. Those wastes contain about 24-27% crude protein, 2-4% fat, 10-14% fiber and 25-35% ash (Biely et al. 1980). Nitrogen value in sheep is similar to soybean meal. Energy value is approximately equivalent to a good hay. Drying conditions partially sterilize the DPW. The feeding mixture of DPW and maize silage to fattening cattle represents another way.

3.4.2. Broiler Litter

Management system is a source of chemical variation. The broiler litter is usually high in fiber with varying amounts of N, Ca, an P. The wastes mentioned are most efficiently utilized by ruminants. When properly processed, they do not impart any objectionable odours or tastes to the final product (Biely et al., 1980). Poultry litter can be fed to dairy cattle up to 25-30% level of dry matter in the ration (Muller, 1981).

3.4.3. Pig Wastes

Pig wastes may be used after mechanical treatments for feeding beef cattle (Muller, 1980) or as a substrate for the production of microbial biomasses. The growth of micro-organisms on agricultural effluents is a possible means of reducing pollutants and of producing protein animal-feed supplements (Miskiewicz et al., 1982; Ringpfeil and Kehr, 1981).

Extensive review of different technologies for animal wastes processing and utilization was published by Muller (1981) as a FAO report.

A detailed description of other technologies and wastes processing is beyond the scope of this paper.

SUMMARY

The continuous increase in world population and protein deficiency in human nutrition indicate a critical need for higher protein production. Better utilization of natural resources, more intensive crop and animal production may be taken as a perspective agricultural way for increasing the protein production in future. The cereals are the most energy productive edible crops. However, they lack essential amino acids. Conversion of cereals to animal protein, which is highly palatable, is preferred in developed countries. As the area of agricultural land is limited and conversion of plant protein through animal husbandry gives a lower yield of protein, the solution consists in increasing amino acid level in crops by means of genetical improvement or after enrichment by pure amino acids or partly by bioproteins.

Technologies for industrial production of bioproteins on a variety of substrates represent net contribution to the world's supply of protein. Bacteria, algae, yeast, and fungi are main organisms for bioprotein production. Nutritional limitation owing to nucleic acid content and presumed texicological aspects predetermine the using of bioproteins mostly as feeds. Especially raw-materials after hydrolysis (sulphite liquor) may be used advantageously for bioproteins production.

Further, the agricultural waste products can be converted to excellent protein food in the chain of animal production. The conversion of ingestible part of plants by ruminants is preferable because competition with human nutrition in edible products is rather low as compared with simple stomached animals. The possibilities of utilization of both lucerne and grass juice and recycling of animal wastes in animal husbandry are being comprehensively discussed, too.

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THE WATERLOO SCP PROCESS: DIRECT CONVERSION OF CELLULOSTIC MATERIALS INTO PROTEINACEOUS FOODS

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1. Process Rationale and Outline

Waste lignocellulosic materials can be upgraded into proteinaceous animal or human foodstuff by mass cultivating microorganisms on them. Previous technologies used indirect conversion by growing yeasts on liquid hydrolysates prepared from the solids. The Waterloo SCP Process uses a fungal organism, *Chaetomium cellulolyticum*, for the direct conversion of the cellulosic components of agricultural and forestry residues by solid-substrate fermentation. The non-carbon nutrient supplements are commercial fertilizer-grade chemicals and/or animal manure, another agricultural residue. Optimal fermentation conditions are in the range: 37^0C with an approx. 10^0C tolerance and pH 5 with an $\pm$ 1.5 tolerance. Specific growth rates of up to 0.25 h^1 can be obtained, the highest for any known direct conversion SCP process. The solid-substrate basis coupled with low pH conditions allow contamination-free operation. An outline of the process is shown in the Figure 1. The core as well as the optimal stages are identified.

Depending on the inherent recalicitrance of the raw material, a mild caustic pretreatment may be required and the pretreatment liquor is concurrently fermented. Typically, grain crop residues, such as straw and cornstover, require a 0.25 - 0.5% w/v NaOH treatment at 121^0C for 15 minutes. Certain preprocessed materials such as Kraft paper pulpmill sludge and sugarcane bagasse pith require no chemical pretreatment. Depending on the process flow conditions, typical particle size conditions are: up to 0.5 cm average diameter for dilute (1-3% w/v) slurry systems and up to 5 cm for dense slurry (solid-state) systems.

2. Economics and Marketing

The process has been successfully tested on a 1 · 3 M^3 - fermenter pilot unit under batch, repeated fed-batch, and continuous (chemostat) conditions. Preliminary feeding trials on mice, rats, poultry and sheep of products made from wheat straw, cornstover and papermill pulp sludge, have indicated good protein nutritional value comparable with soymeal, and no toxic or terogenetic effects. Sensitivity analyses of the process indicate that it is economically feasible for a wide range of industrial and semi-industrial scenarios in several countries, both developed and developing ones. To date, licensing rights for the use and sale of the Waterloo process has been contracted out to three organizations: Envivocon Ltd., Vancouver, Canada; Innotech Inc., Becanson, France and the Provincial

Government of Novi Sad, Yugoslavia. Other contracts are under negotiation.

The tables summarize the amino acid profile of the protein product and the profitability as DCFR (discount cash flow based on the selling price of soybean meal and normal industrial financing) for minimum economic plant sizes in typical Canadian scenarios.

The fungal biomass concentration in the product varies depending on the amount of unutilized cellulose and/or lignin left in it as diet roughage material. In terms of the sulphur-containing amino acids and nucleic acid content, the Waterloo product is better than soybean meal and fodder yeast.

In terms of the economics, it is evident that the basis of renewable and waste-residue raw materials allows the Waterloo process to be more attractive than the existing processes which rely on petroleum resources. As a result of the energy crisis, European SCP production plants based on non-renewable feedstocks have been closed or are about to be closed, e.g. BP (France, UK, Italy), Kangefugi (Italy) based on paraffins, and ICI (UK) based on methanol. So-called village-level SCP technology concepts are as yet unrealized anywhere.

Table 1. Profile of essential amino acids (% DM protein) in the Waterloo fungal SCP and other protein products

Amino Acid	FAO Ref.	Soybean Meal	Waterloo SCP	Fodder Yeast
Isoleucine	4.2	4.2	4.7	5.3
Leucine	4.8	7.7	7.5	7.0
Lysine	4.2	6.4	6.8	6.7
Methionine + Cystine	4.2	2.2	2.6	1.9
Phenylalanine	2.8	4.7	3.8	4.3
Threonine	2.6	3.6	6.1	5.5
Tryptophan	1.4	1.7	N.A.	1.2
Tyrosine	2.8	2.7	3.3	3.3
Valine	4.2	4.4	5.8	6.3

Table 2. Estimated minimum economical plant sizes for the bioconversion of agricultural wastes (e.g. cornstover, straw, manure) into WAT SCP quantities expressed on dry weight basis in tonnes/day (t/d).

(a) NPK from fertilizer. Dried product containing 73% DM SCP

DCFR (%)	ROI (%)	Product (t/d)	Straw (t/d)	Fertilizer (t/d)	Capital (dollars)	Operating (dollars/yr)
10	18	1.9	2.6	0.13	310.700	228.200
20	29	2.5	3.5	0.18	374.300	268.900
30	41	3.6	4.9	0.25	465.500	332.000

(b) NPK from manure anaerobic digester to meet SCP product synthesis requirement. Dried product containing 60% DM SCP.

DCFR (%)	ROI (%)	Product (t/d)	Straw (t/d)	Fertilizer (t/d)	Capital (dollars)	Operating (dollars/yr)
10	18	1.9	1.9	3.6	316.300	194.100
20	29	2.5	2.5	4.8	379.900	222.700
30	41	3.5	3.5	6.6	469.300	265.700

Table 3. Estimated minimum economical plant sizes for the bioconversion of forestry wastes (e.g. Kraft pulpmill clarifier sludge) into WAT-SCP, NPK supplements from fertilizer. Product contains 56% DM SCP.

DCFR (%)	ROI (%)	Product (t/d)	Sludge (t/d)	Fertilizer (t/d)	Capital (dollars)	Operating (dollars/yr)
10	17	3.1	4.3	0.12	639.400	299.900
20	28	4.8	6.6	0.19	837.500	388.500
30	40	8.0	11.0	0.32	1,159.200	548.000

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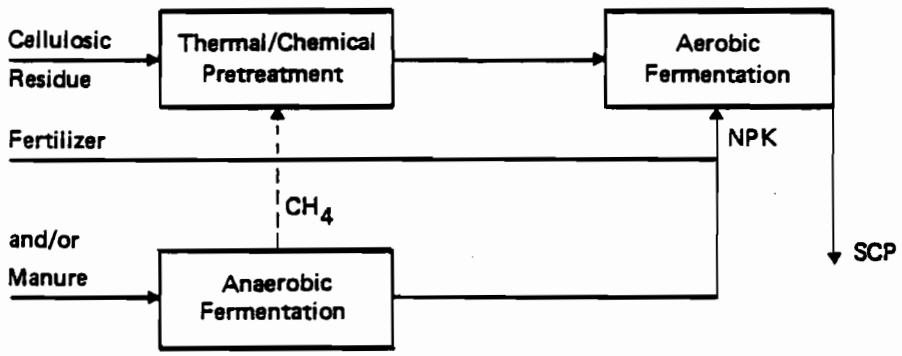

Generalized outline of the Waterloo bioconversion process for SCP production from agricultural or forestry wastes.

THE ROLE OF BIOSYNTHETICAL AMINO ACIDS IN THE MODERN FERMENTAION INDUSTRY

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1. Introduction

The discovery of an L-glutamic acid producing micro-organism by Kinoshita et al. in our company in 1957 contributed not only to the establishment of fermentative production of L-glutamic acid but also to opening the way to fermentative production of almost all of the essential amino acids. The annual production of amino acids in Japan has increased to the level of beyond 300 million dollars. Hence, the importance of amino acids in the modern fermentation industry lies in being supplied not only with a variety of the fine compounds for pharmaceutical uses but also with large amounts of nutritionally valuable compounds for foods and feedstuffs at moderate prices.

The research and development of the microbial production of amino acids have been primarily directed to

(1) obtaining potent amino acid producing micro-organisms by selection from natural sources and by mutation by applying the knowledge of metabolic control,

(2) utilizing some precursors and intermediates in the biosynthetic pathway of the corresponding amino acids, and

(3) applying the enzymatic conversion of chemically derived compounds.

During the course of this development, the selection of carbon sources as the principal raw materials for the fermentation has also played an important part of the research. Even now, efforts are being made to replace agriculturally derived materials by more easlily available sources such as methanol and other inexpensive chemicals. In addition, the use of recombinant DNA technology is recently directed intensively to improving the yield of amino acids.

In this paper, some recent advances in the fermentative production of amino acids in Japan will be described under four headings; *de nove* production, production from some intermediates, enzymatic conversion of chemical substances and application of recombinant DNA technology.

Table 1. Output of Amino Acids in Japan (1980)

Amino acids	Production methods*	Amounts (t/year)**
L-Alanine	E,C	130
DL-Alanine	C	700
L-Arginine-HCl	F	400
L-Aspartic acid	E	250
L-Asparagine	Ex	30
L-Citrulline	F	10
L-Cysteine·HCl	Ex, E	350
L-Cysteine	Ex	40
Glycine	C	3,500
L-Glutamate (Na)	F	89,000
L-Glutamine	F	500
L-Histidine·HCl	F	200
L-Isoleucine	F, Ex	150
L-Leucine	F, Ex	150
L-Lysine·HCl	F, E	20,000
DL-Methionine	C	23,000
L-Methionine	C, E	150
L-Ornithine	F	50
L-Phenylalanine	F, E, C	150
L-Proline	F	100
L-Serine	Ex, C, F	40
L-Threonine	F, C	160
L-Tryptophan	F, E	200
L-Tyrosine	Ex	50
L-Valine	F	150

* F; Fermentation, C; Chemical synthesis, E; Enzymatic conversion, Ex; Extraction from natural sources.

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2. *De Nove* Production of Amino Acids

2.1. Production by Wild Strains

Amino acids efficiently produced by wild strains are limited to a few compounds on amphibolic metabolic pathways. Alanine (Kitai, 1972), L-glutamic acid, L-glutamine (Nakanishi, 1975), or L-valine (Uemura et al., 1972), are producible by wild strains isolated from various environments.

L- Glutamic acid. The discovery of a potent L-glutamic acid producer, *Corynebacterium glutamicum* (Kinoshita et al, 1957), stimulated further screening studies for other micro-organisms. (Nakayama, 1982) Up to now, various bacterial strains have been isolated and identified as species belonging to *Micrococcus*, *Corynebacterium*, *Brevibacterium*, *Arthrobacter* or *Microbacterium*. Comparative studies of conventional taxonomy and chemotaxonomy demonstrated that all of these micro-organisms were closely related (Yamada, et al 1970). Furthermore, in the studies on DNA-homology of glutamic acid producer strains, 9 strains of them, which were efficient producers from carbohydrate sources,

were shown to be highly homologous with *C. glutamicum* as shown in Table 2.

Table 2. DNA-Homology in Glutamic Acid Producing Stains (Nakayama, 1982b).

Strains	DNA-Homology*
C. glutamicum ATCC 13032	100.0
C. acetoacidophilum ATCC 13870	76.2
B. lactofermentum ATCC 13655	102.1
B. divaricatum ATCC 14020	93.4
B. sacchalolyticum ATCC 14066	77.3
B. roseum ATCC 13825	73.5
B. immariophilum ATCC 14068	76.2
B. ammoniagenes ATCC 13745	75.6
M. ammoniaphilum ATCC 15354	78.4

C : *Corynebacterium*

B : *Brevibacterium*

M : *Mycobacterium*

* DNA-homology to *C. glutamicum* ATCC 13032

Yields of L-glutamic acid fermentation by various bacterial strains using different carbon sources are shown in Table 3. The industrial production in Japan is performed using carbohydrate sources such as glucose or molasses. In these cases, biotin exerts critical effects on L-glutamic acid production. Only when its limiting amount (1 to 5 $\mu g/l$) is present in the medium does appreciable production of L-glutamic acid occur. The function of biotin was proposed to be in the regulation of cell permeability in the cell membrane to make a value higher than 1; only in this case the cells excreted large amounts of L-glutamic acid to the outside of the cells. In the process used a molasses medium containing sufficient biotin, with the addition of penicillin or its analogous substances capable of inhibition the cell envelope biosynthesis, or the addition of some surface active agents, brought about a remarkable production of L-glutamic acid as a result of the alteration in permeability of the biotin-sufficient cells. On the other hand, the similar relationship between glutamic acid production and membrane phospholipid synthesis was demonstrated using a glycerol-requiring mutant of *Corynebacterium alkanolyticum* or an oleic acid-requiring mutant of *Brevibacterium thiogenitalis*. These mutants produced significant amounts of L-glutamic acid, regardless of biotin concentration, only by controlling glycerol or oleic acid in the medium to suboptimal concentration for the cell growth. The relation of L-glutamic acid excretion to the membrane permeability is illustrated in Fig. 1.

Table 3. Fementative Production of L-glutamic acid

Carbon source	Yield (g/l)	(%)	Micro-organism	published age
Glucose or Molasses	38	38	*C. glutamicum*	1957
	50	50	*B. flavum*	1962
	29	29	*M. ammoniaphilum*	1963
Acetic acid	98	48	*B. flavum*	1971
	51	51	*B. thiogenitalis*	1972
Ethanol	59	66	*B. sp.*	1969
Propylene glycol	27	27	*Ba. megaterium*	1962
n-Paraffin	62		*A. paraffineus*	1971
	84		*C. hydrocarboclastus*	1972
	72		*C. alkanalyticum*	1972
Benzoic acid	80	80	*B. sp.*	1972
Xylose	5	10	*B. pentosoaminoaudicum*	1960

A; Arthrobacter, B; Brevibacterium, Ba; Bacillus,

C; Corynebacterium, M; Microbacterium.

The morphological studies showed that a biotin-deficient medium gave elongated or swollen cells which were very different from the cells in a biotin-sufficient medium. This finding supported the alteration of cell permeability. The fine structure of the cell envelope in *C.glutamicum* was studied using the electron microscope by the freeze-etching method. As represented in Fig. 2, significant differences were observed between the biotin-sufficient and deficient cells; regularly arrayed small particles were patched in the outer concave surface and many blebs were formed on the mucopeptide layer in the biotin-deficient cells (Ochiai, et al, 1982).

The mutation researches of glutamic acid producing strains have been extensively carried out. The yield improvements were achieved by mutants such as temperature-sensitive strains, drug-registant strains and defective strains of some enzymes on TCA cycle (Kikuchi et al, 1982).

2.2. Production by mutant strains

Generally, biosyntheses of terminal amino acids are under strict metabolic controls such as feedback inhibition and repression in order to avoid wasteful production. Therefore, it is required for extracellular production of desired amino acids to change cellular metabolisms and regulatory systems to allow overproduction of amino acids. The first attempt was achieved by induction of auxotrophic mutants which required one or more amino acids located at more terminal positions in the synthetic pathway involving the desired amino acid. By addition of suboptimal amounts of such amino acids required for the growth of the mutants, the feedback inhibition or repression by these amino acids could be released to lead to accumulation of the desired amino acids. The second was by regulatory mutants such as structural analog-resistant strains. The industrial production of amino acids at present is conducted by use of the mutant derived from combination of these two mutation methods, as shown in Table 4 (Nakayama, 1982a). Among them, some typical examples are explained below.

Table 4. Amino Acid Production using *C. glutamicum* Mutant Strains (Nakayama, 1982a).

Amino Acid Produced	Type of Strain	Main Contributors to Production
L-Valine	Auxotrophic mutant	Ile^-,Leu^-
L-Homoserine		Thr^-
L-Lysine		$Homoserine^-$, Thr^-
L-Ornithine		$(Arg/Cit)^-$
L-Citrulline		Arg^-
L-Leucine		$Aminoacids^-$
L-Proline		$Base^-$, Arg^-, Ile^-, His^-
L-Arginine	Regulatory mutant	$Arg-analog^R$
L-Histidine		$His-analog^R$
L-Threonine	Auxotrophic regulatory mutant	Met^-, $Thr-analog^R$, $Lys-analog^R$
L-Isoleucine		Met^-, $Thr-analog^R$ $Lys-analog^R$, $Ile-analog^R$
L-Tryptophan		Phe^-, Tyr^-, $Phe-analog^R$ $Tyr-analog^R$, $Trp-analog^R$
L-Tyrosine		Phe^-, $Tyr-analog^R$ $Phe-analog^R$
L-Phenylalanine		Tyr^-, $Phe-analog^R$ $Tyr-analog^R$
L-Lysine		$Homoserine^-$, Leu^- $Lys-analog^R$

L- Lysine, the necessity of L-lysine supplementation to various foods and feedstuffs which are low in lysine content has been stressed for parts of the world. The fermentative production of L-lysine was first established by Kinoshita et al. in 1958 (Kinoshita, 1978), by use of the auxotrophic mutant of *C. glutamicum*, requiring L-homoserine or L-threonine and L-methionine. Figure 3 shows briefly the mechanism for L-lysine production by this auxotroph. The existence of both L-lysine and L-threonine reveals "concerted feedback inhibition" to aspartokinase, the first enzyme in biosynthetic pathway of L-lysine, but the L-homoserine auxotroph which is defective in homoserine dehydrogenase would accumulate L-lysine extracellularly by keeping the L-threonine level in the medium low. In this fermentation, the level of biotin which is a growth factor of *C. glutamicum* should be kept higher than 30 μg/liter in the medium.

Fermentative production of L-lysine was also achieved by use of a regulatory mutant, S-(β-aminoethyl)-L-cysteine (AEC) resistant mutant of *Brevibacterium flavum*. This was explained by the possibility that aspartokinase was modified by the lysine-analog to become insensitive to the concerted feedback inhibition by L-lysine and L-threonine. Further attempts have been made to induce the mutants resistant to some other lysine-analogs such as α-chlorocaprolactam and γ-methyl lysine. A mutant of *C. glutamicum*, which was the auxotroph of homoserine and leucine as well as an AEC-resistant strain, provided higher yield of L-lysine as compared with the parent auxotroph. When the culture condition was optimized for these improved strains, the production yield

of L-lysine was reported to reach up to 50% of the amounts of the carbohydrate raw material used (Tosaka et al, 1982)

L- Threonine; L-Threonine is also one of the limiting amino acids in cereal proteins, hence, useful for animal feed additives as well as an ingredient of infusion solution.

L-threonine production was extensively studied using various types of *E. coli* auxotrophs (Nakayama, 1982a; Kase et al 1972; Huang 1961).The isoleucine-revertant strain derived from *E. coli* mutant requiring for diaminopimelate, methionine and isoleucine produced about 14g of L-threonine per liter of the medium containing 7.5% fructose. The *C. glutamicum* mutant which was resistant to both α-amino-β-hydroxy valeric acid (AHV, a threonine-analog) and a lysine-analog (AEC) was derived from the methione auxotrophs (Kase et al, 1974). This strain was more favorable to practical use, because more than 14 g of L-threonine was produced from 10% of glucose. In addition, some AHV-resistant mutants were obtained from the auxotrophs of *Brevibacterium* (Nakamori et al, 1972). and *Serratia marcescens* (Komatsubara et al, 1978). The *S. marcescens* mutant which was resistant to both AEC and ethionine was reported to produce L-threonine with the yield of 45.4 g/1 (Chibata et al, 1981).

L- Tryptophan and *L- phenylalanine*; These are also essential amino acids, and L-tryptophan is one of the limiting amino acids in cereal proteins. Therefore, these amino acids are particularly useful not only as additives to animal feeds but also as ingredients of infusion solution.

Production of these amino acids by the direct fermentation from carbohydrate raw materials was not easy because of the very complex and strict regulatory mechanisms in the biosynthesis of these amino acids. The research group in our company, however, succeeded in the induction of various auxotrophic and analog-resistant mutants of *C. glutamicum*, which were capable of producing considerable amounts of these amino acids. The regulatory mechanism as shown in Fig. 4 was proved to be released in these mutants. As summarized in Fig. 5, a strain PX-115-97 accumulated 12 g of L-tryptophan per liter of a medium containing 10% sucrose (Hagino et al, 1975). In another study, the addition of resistance to p-fluorophenylalanine (PEP) and p-aminophenylalanine (PAP) to a tyrosine auxotroph of *C. glutamicum* resulted in an increase of L-phenylalanine production up to 9.5 g/l (Hagino et al, 1978). Furthermore, an L-tyrosine auxotroph of *B. flavum*, which was resistant to PFP and 5-methyltryptophan and also sensitive to decoynine, was reported to produce 25 g/l of L-phenylalanine.

Other Amino Acids: The production of L-arginine has been studied using regulatory mutants of a variety of micro-organisms (Kisumi et al, 1977a). From an L-isoleucine auxotroph of *C. glutamicum*, which was sensitive to D-serine and resistant to arginine hydroxamate plus D-arginine, was induced an L-isoleucine-revertant strain, which was found to produce 25 g of L-arginine per liter of the medium containing 15% sugar as molasses (Nakayama et al, 1972). The production by a guanine-requiring and 2-thiazolealanine-resistant strain of *B. flavum* was also reported. Improvement of an L-arginine producing strain using a transduction method was applied to *S. marcescens* (Kisumi et al, 1978)

L-Histidine was produced by a mutant of *C. glutamicum*, resistant to 2-thiazolealanine or 1,2,4-triazole-3-alanine (Araki et al, 1974). Further addition of resistance to structural analogs of purine, pyrimidine and tryptophan to the above mutant gave the promoted productivity of the amino acid. The mechanism related to such overproduction was explained as desensitization and derepression of phosphoribosyl-ATP-pyrophosphorylase, the first enzyme of histidine biosynthetic pathway. Many other analog-resistant mutants have been

induced from *B. flavum* and *S. marcescens* (Kisumi et al, 1977b) as L-histidine producing strains.

3. Production of Amino Acids from Intermediates

Microbial production of amino acids from intermediates has been studied, because this is another useful method for avoiding any feedback control mechanism. In some cases, this method was very effective and has been successfully applied to the production of various amino acids as shown in Table 5. Based on this method, L-serine has been produced on a industrial scale. Microbial conversion of glycine into L-serine was studied by use of a mutant of *Nocardia butanica*, which was defective in the enzyme for L-serine degradation and multi-resistant to glycine hydroxamate, ethionine and methionine sulfone. The production was stimulated efficiently by addition of tribasic magnesium phosphate into the medium (Tanaka et al, 1980).From 35 g/l of glycine added was formed 19 g/l L-serine in 6 days. A temperature-sensitive mutant of *Pseudomonas* MS 31, a facultative methylograph, produced L-serine from glycine in a high yield (Morinaga et al, 1981). The conversion rate increased significantly when the temperature was shifted from a permissive (30°C) to non-permissive state (38-40°C). *Hyphomicrobium* sp. (Yamada, private communication) and *Sarcina aliva* (Ohmori et al, 1979) were also used for the conversion.

Table 5. Amino Acids Produced from Precursors

Amino Acid Produced	Precursor
L-DOPA	L-Tyrosine
L-Glutamic acid	α-Ketoglutarate
L-Histidine	L-Histidinol
L-Homoisoleucine	L-Isoleucine
L-Isoleucine	D-Threonine
	α-Aminobutyric acid
	DL-α-Hydroxybutyric acid
	DL-α-Bromobutyric acid
L-Methionine	L-Hydroxy-4-methyl thiobutyric acid
L-Norleucine	L-Norvaline
L-Norvaline	α-Amino butyric acid
	D-Threonine
L-Serine	Glycine
	Glyceric acid
	L-Threonine
L-Threonine	L-Homoserine
L-Tryptophan	Anthranilic acid
	Indole

In the L-isoleucine fermentation by *S. marcescens*, D-threonine was added into the medium as a precursor to avoid the inhibition of L-threonine dehydratase by L-threonine, because D-threonine was convertible to α-ketobutyrate, a precursor of L-isoleucine, by D-threonine deaminase which was not inhibited by L-isoleucine. Besides, α-Aminobutyrate and α-hydroxy butyrate were also used as precursors of L-isoleucine (Shimura, 1972).

4. Enzymatic Conversion

The enzymatic method implies microbial transformation with intact cells or enzymes extracted from the cells. Some chemically derived compounds or microbial products are convertible to the amino acids using various kinds of microbial cells or enzymes as shown in Table 6. This method is useful for the industrial production of amino acids if the starting materials are available at inexpensive prices, and also expected to make an interdisciplinary field between biochemistry and synthetic chemistry with the active cooperation of both fields. L-Aspartic acid, L-alanine and L-lysine have been produced in the industrial scale by this method. *E. coli* cells as an aspartase source converted fumaric acid to L-aspartic acid with the conversion yield of 99% in molar ratio. Particularlly, either culture broth or immobilized cells of *E. coli* was employed for this production process.

Table 6. Amino Acids Produced by Enzymatic Conversion Methods

Amino Acid	Substrates	Enzyme Participated
L-Alanine	L-Aspartic acid	Aspartate β-decarboxylase
L-Aspartic acid	fumaric acid, NH_3	Aspartase
L-Citrulline	L-Arginine	Arginine deiminase
L-Cysteine	DL-2-Amino-2-thiazoline-4-carboxylic acid (DL-Thiazolidine-4-carboxylic acid)	L-ATC hydrolase S-Carbamyl-L-cysteine-hydrolase, Racemase,
	β-Chloro-L-alanine, Na_2S	Cysteine desulfhydrase
L-DOPA	Pyrocatechol, Pyruvate, NH_3	β-Tyrosinase
	Tyrosine	Tyrosinase
L-Glutamic acid	DL-Hydantoin-5-propionate	L-Glutamate hydrolase
L-Lysine	DL-α-Amino-ε-caprolactam	ACL racemase L-ACL-hydrolase
	Diaminopimelic acid	DAP-decarboxylase
	5(4-amino butyl)hydantoin	Hydrolase
L-Phenylalanine	DL-Phenylalanine-hydantoin	Hydrolase
	Cinnamic acid, NH_3	L-Phenylalanine ammonialyase
L-Tryptophan	DL-Tryptophan-hydantoin	Hydrolase
	Indole, Pyruvate, NH_3	Tryptophanase
	Indole, Serine	Tryptophanase
L-Tyrosine	Phenol, Pyruvate, NH_3	β-Tyrosinase

L-Alanine was producible from L-aspartate by aspartate-β-decarboxylase in *Pseudomonas dacunae* with a considerable yield (Chibata et al, 1965). The immobilized method of the intact cells or the crude enzyme was also applied in this reaction.

A part of the output of L-lysine in Japan is derived from enzymatic conversion of DL-α-amino-ε-caprolactam (DL-ACL) (Fukumura, 1977). Using the cells of both micro-organisms, *Cryptococcus laurentii* as an L-ACL hydrolase source and of *Achromobacter obae* as a DL-ACL racemase source, DL-ACL was converted to L-lysine with the yield of 99.8%. At present, annual production increases to 3,000 t which is estimated approximately one-seventh of total production of L-lysine in Japan.

There were many investigations on the production of L-tryptophan from chemically synthesized substrates. When idole, pyruvate and ammonium acetate were added into the culture broth of *Proteus rettgeri* in the presence of inosine and trace amounts of pyridoxal phosphate, L-tryptophan was formed in 48 hours with yield of 96% from idole added (Yamada, et al, 1974). Addition of inosine was a sophisticated technique because L-tryptophan produced was removed from the reaction system as an isoluble complex with isosine. Other indole derivatives were also converted to corresponding tryptophan analogs by the above cells. The washed cells of *Achromobacter liquidum* also produced L-tryptophan from L-serine and indole. In another experiment, the cells of *Flavobacterium aminogenes* hydrolyzed hydantoin derivatives of DL-tryptophan to L-tryptophan (Sano et al, 1977a) with nearly 100% yield in molar ratio.

N-Carbamyl-D-p-hydroxy phenylalanine was obtained from DL-5-hydroxyphenylhydantoin using hydantoinase of *Pseudomonas striata*. The product was converted chemically to D-p-hydroxyphenylglycine, a component of a semisynthetic penicillin (Yamada et al 1978a).

L-Cysteine was synthesized enzymatically from β-chloroalanine and sodium sulfite by *Enterobacter cloacae* cells as a cysteine desulfhydrase source (Yamada et al 1978b). *Pseudomonas thiazolinophilum* also produced L-cysteine from DL-2-aminothiazoline-4-carboxylate (ATC) assumably by the participation of three enzymes such as ATC-racemase, ATC-hydrolase and S-carbamylcysteine hydrolase (Sano et al 1977b). In this study, DL-thiazolidine-e-carboxylic acid was also employed as a substrate of cysteine formation by *Pseudomonas* sp. or *Trichoderma viride*.

Using *Erwinia herbicola* cells as a β-tyrosinase source, L-tyrosine or L-Dopa was synthesized from phenol or pyrocathecol in the presence of pyruvate and ammonia (Enei, et al, 1972). L-Phenylalanine was produced from cinnamic acid with L-phenylalanine ammonia-lyase in *Rhodotorula glutinis* (Yamada et al 1981).

5. Application of Recombinant DNA Technology

Mutation, an essentially random process, has been used successfully to improve the productivity of amino acids as mentioned above. Recombinant DNA technology is likely to be applied to organisms used in amino acid production, as well. An earlier attempt was made for yield improvement of L-threonine by *E. coli*. (Debabov et al 1981; Debabov 1982) has succeeded in obtaining *E. coli* K-12 strains capable of L-threonine overproduction, in which a threonine operon (the entire operon or its parts) was introduced through the transformation of hybrid plasmids with pBR 322 and amplified to more than 50 copies. The genes involved in sucrose utilization have been also introduced to the above strain. The optimization of cultivation conditions allowed to produce up to 55 g/l of L-threonine.

Almost the same attempt was made in Japan (Momose et al, 1980). The chromosomal DNA fragment was extracted from *E. coli* mutant BIM 4 which was resistant to AHV and required L-isoleucine, L-methionine, L-proline and thiamine for the growth, and introduced into an L-threonine--requiring mutant of *E. coli* after combining with a plasmid pBR 322. A hybrid plasmid PAJ-294 including threonine operon was extracted from the threonine revertant thus obtained and incorporated again into appropriate recipients of *E. coli*. Such the transformant strain produced L-threonine with the yield of 4-fold of the parent strain. The similar methods were applied to the production of many other amino acids by *E. coli*.

For the yield improvement of L-tryptophan, tryptophan operon was introduced to *E. coli* W 3110 which deleted tryptophan operon and was deficient in

tryptophan repressor and tryptophanase (Aiba, et al, 1980). The plasmid used here was characterized by having entire tryptophan operon and tretracycline-resistant gene. The transformant which was proved to be desensitized to an end product inhibition by L-tryptophan produced extracellularly 5.5 g/l in 24 hr when anthranilic acid was fed to the medium at a rate of 50 mg/l/hr.

More extensive yield improvement is unlikely to depend on *E. coli* because this organism is rarely used industrially. It is therefore required to develop the alternative host-vector systems to *E. coli* in extensively used producer organisms such as *Corynebacterium glutamicum*. There are however several factors which will inevitably slow the rate of progress in this field. These include the limited knowledge of molecular genetics of these producer strains, diversity of the organisms used and complexity of biosynthetic pathways of amino acids. An early attempt will be made to multiply a few enzyme functions which are proved to be limited in the biosynthetic pathways.

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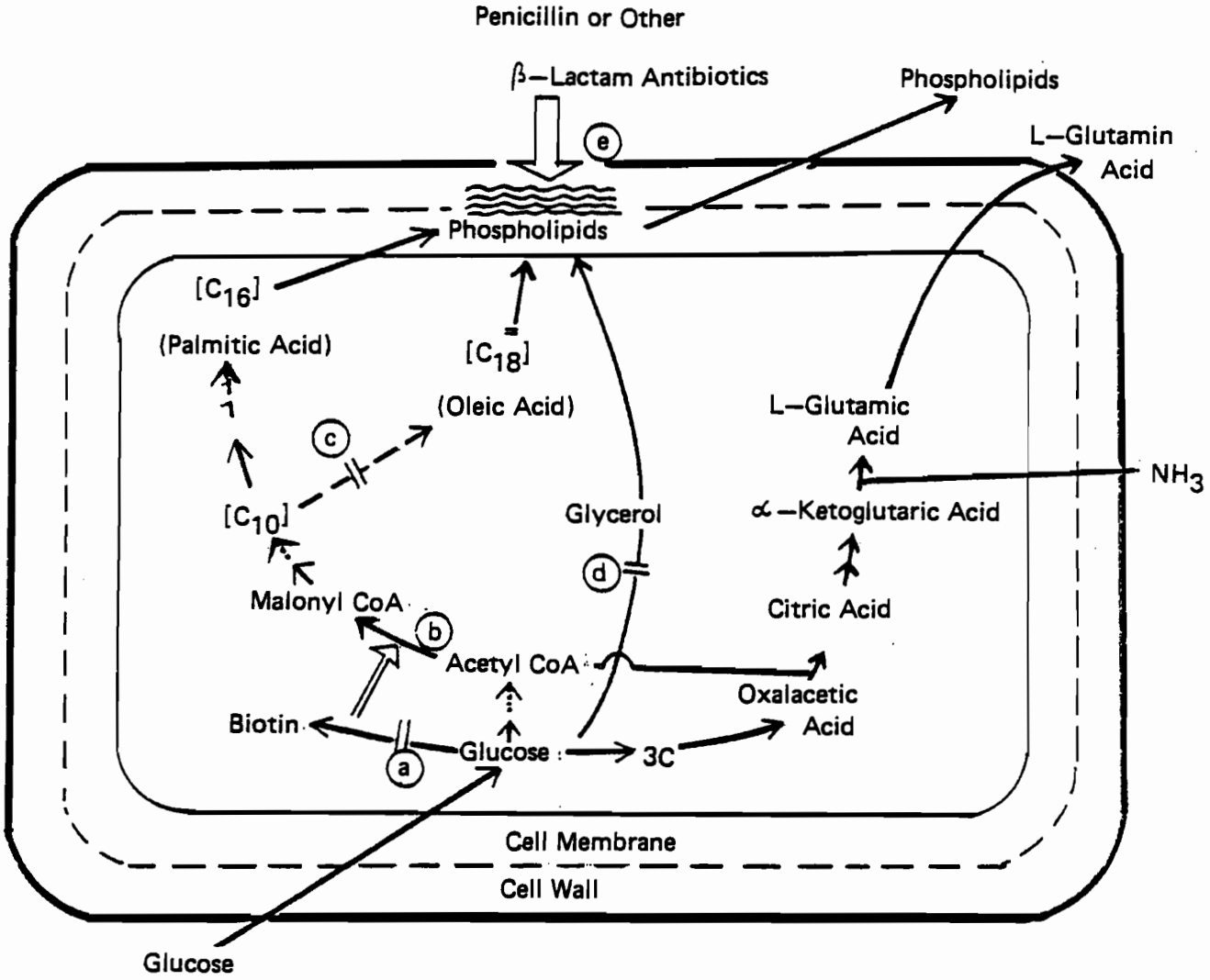
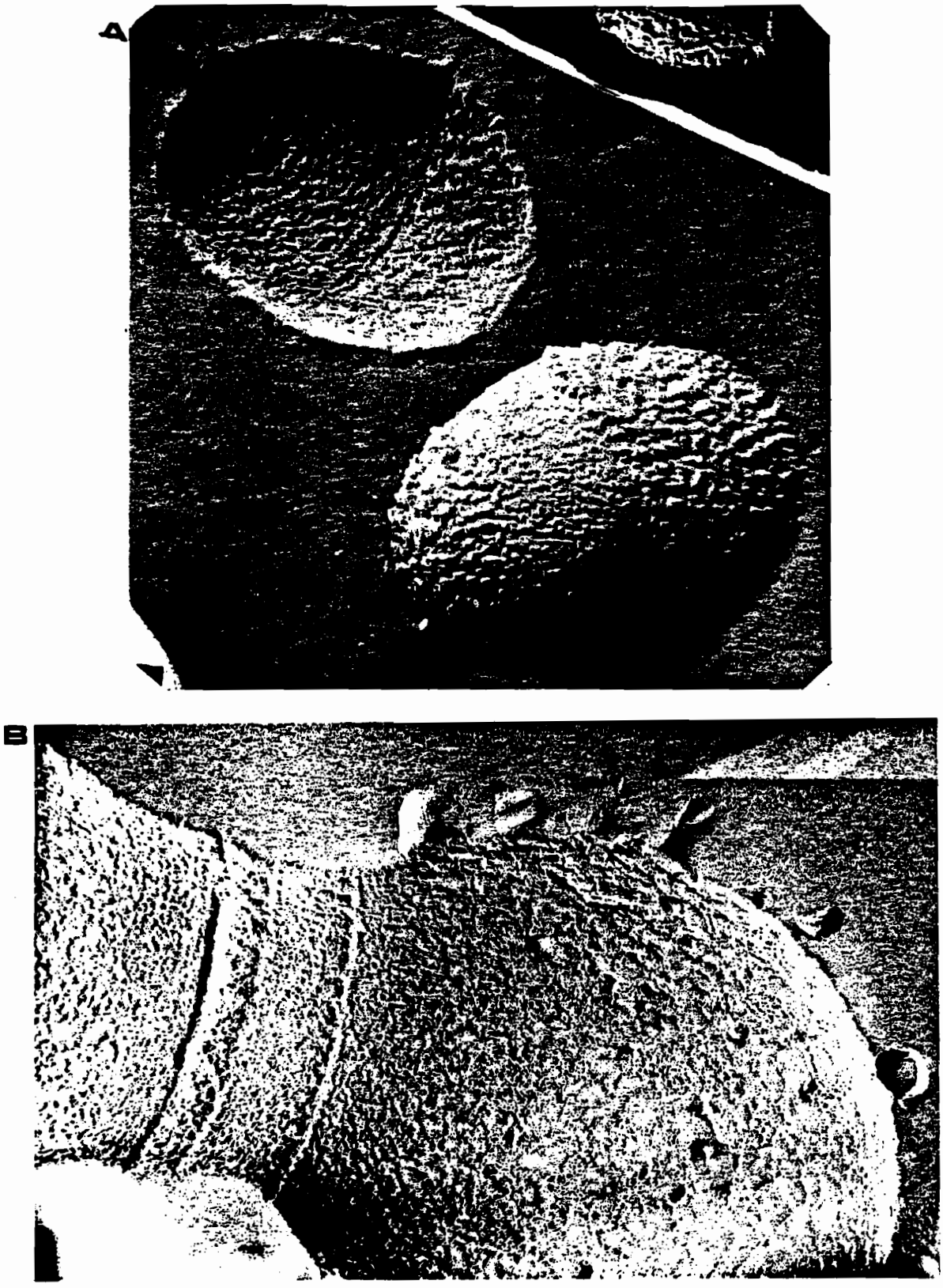


ⓐ : Deficiency of biotin biosynthesis
ⓑ : Acetyl CoA carboxylase
ⓒ : Blocking site in an oleic acid requiring mutant
ⓓ : Blocking site in a glycerol requiring mutant
ⓔ : Inhibition of cell wall synthesis by Penicillins

Fig. 1. Relationship between L-Glutamic Acid Excretion and Cell Permeability



A : Biotin–Sufficient Cells (x 52000)
B : Biotin–Deficient Cells (x 56000)

Fig.2 Structures of cell envelope in *C. glutamicum*.

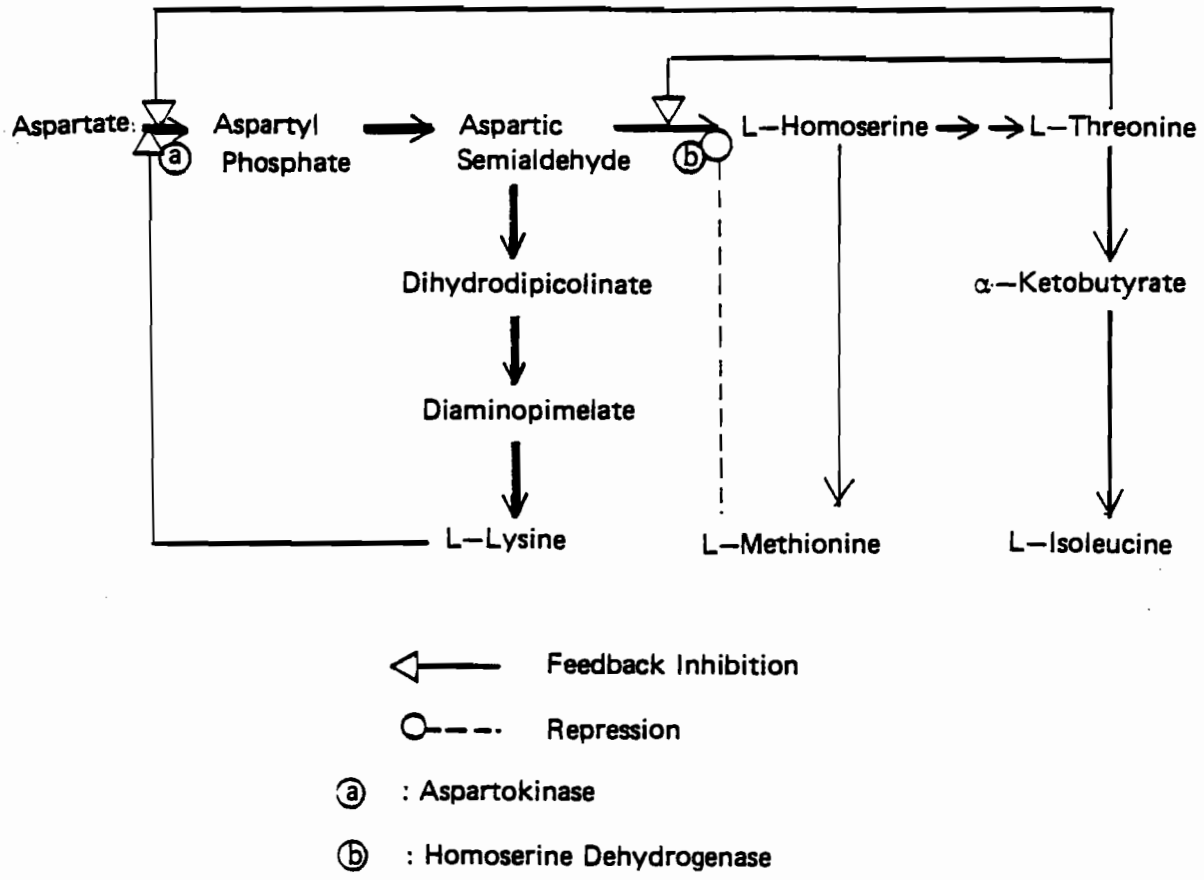
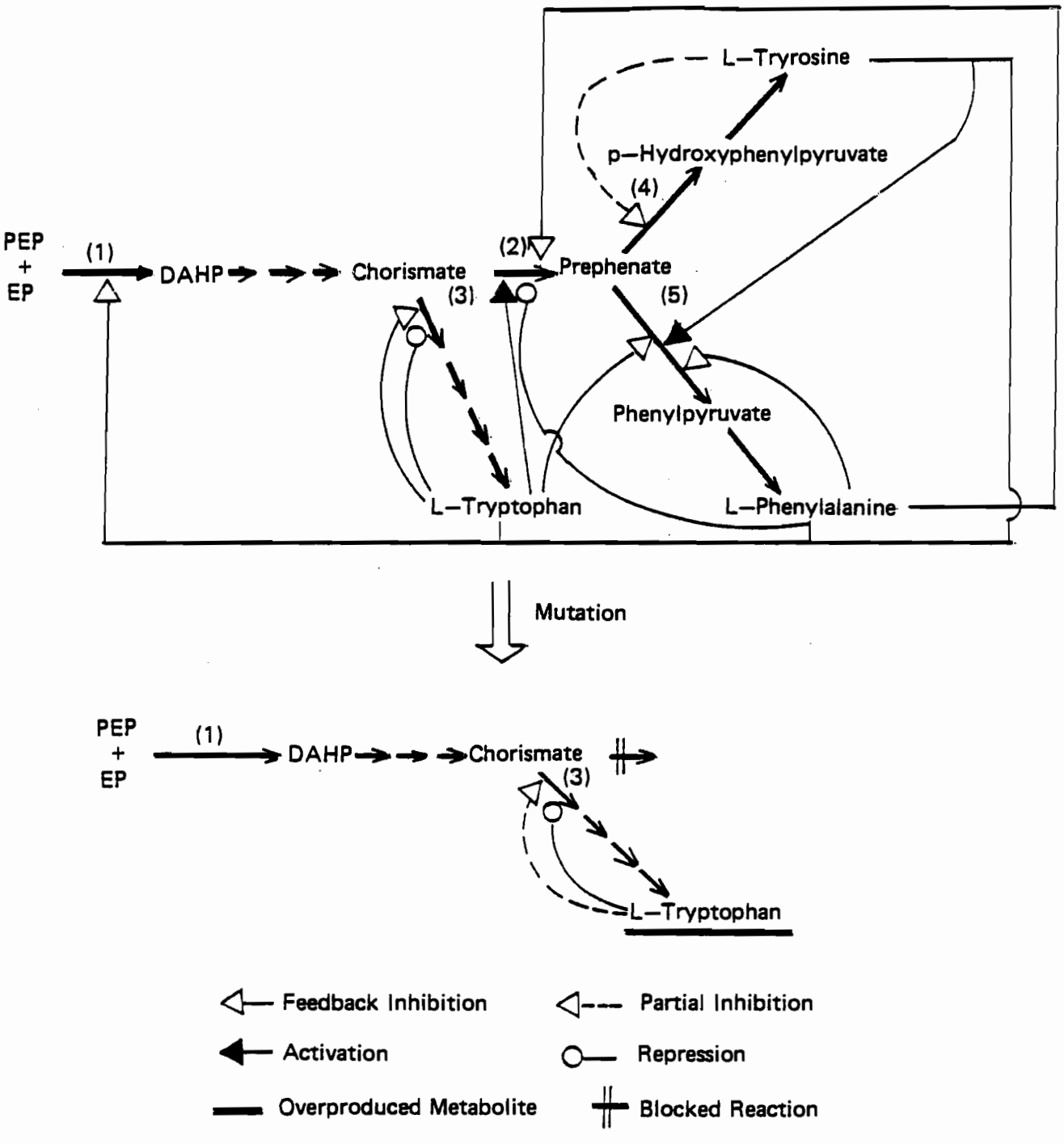


Fig. 3. Biosynthetic Pathway of L-Lysine and Its Regulation Mechanism



(1) DAHP Synthetase, (2) Chorismate Mutase, (3) Anthranilate Synthease,
(4) Prephenate Dehydrogenase, (5) Prephenate Dehydratase

PEP: Phosphoenolpyruvate, EP: Erythrose–4–Phosphate,
DAHP 3–Deoxy–D–Arabinoheptulosonic Acid–7–Phosphate

Fig. 4. Control of Aromatic Amino Acid Biosynthesis in C. Glutamicum and Deregulation in Tryptophan-producing Mutant

	L–Tryptophan Produced (mg/ml)
KY 9456 (Phe^-, Tyr^-)	0.15
↓ 5–MT^R, Trp Hx^R	
↓ 6–FT^R, 4 MT^R	
4 MT–11	4.9
↓ PFP^R	
PFP–2–32	5.7
↓ PAP^R	
PAP–126–50	7.1
↓ Tyr HX^R	
Tx–49	10.0
↓ Phe Hx^R	
Px–115–97	12.0

5MT: 5–Methyltryptophan
6FT: 6–Fluorotryptophan
PFP: p–Fluorophenylaline
PheHx: Phenylali Phenylalanine hydroxamate
Trp Hx: Tryptophan hydroxamate
4MT: 4–Methyltryptophan
PAP: p–Aminophenylalanine
PyrK PyrHx: Tryosine hydroxamate

Fig. 5. Process of Improvement of L-Tryptophan Producing Strains

ANIMAL FEED FROM EFFLUENTS AND SEWAGE

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Abstract

Protein from a variety of industrial effluents and activated sewage sludge has been isolated and analysed for amino acids. In general, the content of essential amino acids indicated a high nutritional value. This has been confirmed by feeding trials in animals, poultry and fish. Good growth rates were obtained with no evidence of toxic effects.

1. Introduction

It has been estimated that between 2 and 5 % of the total carcase protein is lost in the effluents from abattoirs and poultry processing plants. In the U.K. this amounts to tens of thousands of tonnes per annum with a potential value at present in the region of UK £ 200 per tonne, for the world as a whole, this loss needs to be multiplied by a factor of about 100. At the present time when world population is increasing rapidly and outstripping food production in many areas such a wastage is hard to justify if the means of preventing it are available.

In the past, various processes have been proposed involving the use of chemical precipitants to remove protein from solution. These have usually employed toxic compounds such as iron salts which results in the precipitated protein being useless for nutritional purposes. The Aquapure effluent treatment process allows the recovery of protein in a completely non-toxic form suitable for feeding to both domestic and farm animals (Grant 1974; and 1975).

Town sewage, industrial effluents and process waste waters are commonly treated by the activated sludge process.

Dried activated sludge contains a high percentage (50-60) of protein. This protein has been shown to have a rich content of the essential amino acids, and in this respect, compares well with casein and egg albumin both of which have excellent nutritional values. The amount of surplus activated sludge produced in the U.K. alone amounts to 220,000 tonnes dry weight per year and represents a potential protein yield of about 100,000 tonnes if it could be processed to give an acceptable product. The market potential for a recovered protein product averaged 295,000 tonnes per month over the period 1975/78 and 271,000 tonnes for eight months of 1979 (source: CSO Monthly Digest of Statistics).

Activated sludge may be regarded as a form of single cell protein (SCP) where the feedstock is available at nil cost. In this respect it may be compared

with SCP made from hydrocarbon feedstock; originally this appeared to be a very attractive proposition but the recent enormous increases in world oil prices have made the economics much less viable whereas economic considerations favour the recovery of protein from surplus activated sludge.

2. Effluent Treatment

The process consists essentially of a flocculation reaction whereby soluble protein together with insoluble suspended protein particles is removed from the effluent, the floc entraps fat globules which are removed simultaneously with the protein. The flocculated protein plus fat is separated from the effluent by air flotation and skimming in the form of a sludge containing up to about 15% total solids. The relative amounts of protein and fat in the separated sludge are dependent on the composition of the effluent. In general, the protein/fat ratio is higher in the case of slaughterhouse effluents than for poultry processing effluents. Where the effluent contains exceptionally large quantities of fat as in the case of cooking and bone degreasing effluents the bulk of fat may be recovered in a separate air flotation stage. It has been found from analytical studies on a large number of effluents that the B.O.D. and C.O.D. (chemical oxygen demand) levels can be reduced by about 70 - 90 % of the initial value with virtually complete removal of fat and suspended solids.

3. Effluent By-products

The protein content of a typical slaughterhouse effluent by-product is given in Table 1. The contents of essential amino acids in two specimens are shown in Table 2 compared with the FAO recommendation for human nutrition, apart from tryptophan which was not determined, the essential amino acid content appeared adequate. A similar analysis for amino acids was carried out on a sample of protein recovered from poultry processing effluent (Table 3).

The nutritional value of recovered meat works effluent protein was determined in a standard feeding trial on chicks. The relative growth rates, feed consumption and feed efficiencies are shown in Table 4.

A further feeding trial on the effluent protein was carried out using pigs. A satisfactory growth rate was obtained over 68 days, when the diet included effluent protein at the 5 % level (Table 5).

Table 1. Composition of by-product recovered from slaughterhouse effluent. (%)

Batch No.	1	2	3	4	5	6	Means
Nitrogen	10.5	11.0	11.3	11.5	11.5	10.6	11.1
Protein	65.5	68.0	70.5	72.0	72.0	65.6	68.9
Total Organics	74.5	78.3	77.6	77.4	76.2	72.7	76.1
Ash	21.8	17.7	18.5	19.1	20.0	23.6	20.1
Moisture	3.7	4.0	3.9	3.5	3.8	3.7	3.8

Table 2. Essential amino acids (g amino acid/16g nitrogen).

Amino acid	FAO*	Egg	Fraction A	Fraction B	Casein
Isoleucine	4.2	6.8	5.5	4.1	7.5
Leucine	4.8	9.0	17.1	15.0	10.0
Lysine	4.2	6.3	8.8	8.5	8.5
Phenylalanine	2.8	6.0	9.9	7.9	6.3
Tyrosine	2.8	4.4	5.5	3.2	6.4
Threonine	2.8	5.0	7.9	4.5	4.5
Tryptophan	1.4	1.7	-	-	-
Valine	4.2	7.4	11.0	8.2	7.7
Sulphur containing:					
Total	4.2	5.4	2.8	3.2	4.3
Methionine	2.2	3.1	2.8	3.2	3.5

* Food and Agricultural Organization "provisional pattern" of essential amino acids for human nutrition: Rome, 1957.

Table 3. Amino acid analysis of protein recovered from poultry processing plant effluent (umoles/100 umoles)

Amino Acid	umolves/100 umoles	Amino Acid	umolves/100 umoles
Aspartic acid	10.8	Threonine	5.1
Serine	7.7	Glutamic	
Proline	4.5	acid	11.5
Alanine	8.9	Glycine	6.9
Valine	7.4	Cystine (half)	1.2
Isoleucine	5.3	Methionine	1.5
Tyrosine	2.3	Leucine	9.1
Lysine	6.6	Phenylalanine	3.8
Arginine	4.9	Histidine	2.3

Tryptophan not estimated.

Table 4. Chick growth, feed consumption and feed efficiency of experiment rations.

	Body weight gain/chick 1-2 weeks	Food Consump-/ tion/chick 1-4 weeks	Feed con-sumption(g) weight gain
A. Ref.ration (casein)	137.0	359	2.62
B. Mm 41	147	400	2.74
C. Mb 43	103	347	3.38
D. Mb 44	100	302	3.02
E. Mm 45	166	413	2.49
F. Mb 46	83	282	3.42
G. Mm 47	141	386	2.73
H. Grass Protein	104	338	3.16
I. Fishmeal 6	169	418	2.48
J. Eff. protein	125	345	2.75

Table 5. Pig trial rations and results

	Effluent by-product %	Whey %	Control %
Barley	42.0	31.2	43.0
Maize	41.75	31.2	43.0
Meatmeal (60%)	11.0	10.0	13.75
Whey Mix (13.4%)	-	26.75	-
Trace Nutrients	0.25	0.25	0.25
Steamed Bone Flour	-	0.6	-
Effluent by-product	5.0	-	-
	100.0	100.0	100.0
Estimated total protein %	17.0	16.9	17.0
Results			
Original liveweight (kg)	14.0	14.4	14.3
68 trial days liveweight (kg)	39.5	33.4	41.6
68 day gain (kg)	25.5	19.0	27.3
Average daily gain (kg)	0.38	0.28	0.40

4. Vegetable Wastes

Vegetable processing effluents may also contain significant amounts of protein which can be recovered by suitable chemico-physical treatment for use in animal or human nutrition. It has been found possible to recover the soluble protein from rice starch factory effluent by flocculation and air flotation in a yield amounting to about 5 % of the weight of the rice processed.

In the case of the palm oil industry vast quantities of centrifugation sludge and sterilizer condensate are produced. By means of flocculation followed by centrifugation or air flotation and drying a product containing about 12% protein and 80% low grade carbohydrate was obtained in a yield of about 4% W/V and possibly useful as ruminant feed.

5. Activated Sludge Protein

The essential amino acid content of activated sludge protein (Table 6) clearly indicates its potential value as an animal or fish feed. Tacon (1976, 1978/79, 1978) has made extensive and detailed studies of activated sludge protein as a component of trout feed. He showed that activated sludge protein could be incorporated into the diet of rainbow trout up to a level of 20% without any apparent harmful effects. The fish took the modified feed readily and there was no significant difference in the specific growth rate or feed conversion. In general, gross body protein increased and fat content decreased with progressive substitution of the control diet and no deleterious effects were observed during the trial. The carcase ash content did not vary significantly between control and experimental groups at the end of the trial period (5 weeks). The only metals showing marked increases in the body tissues were aluminium and iron whereas the contents of the toxic elements cadmium and lead did not differ significantly from the controls.

Table 6. Amino acid content of activated sludge protein (g amino acid/16 g N).

Amino Acid	g amino acid/16g N	Amino Acid	g amino acid/16g N
Lysine	7.8	Glycine	7.3
Histidine	2.9	Alanine	6.0
Arginine	10.0	Valine	4.3
Aspartic acid	11.0	Methionine	2.9
Threonine	6.4	Isoleucine	4.1
Serine	5.4	Leucine	10.6
Glutamic acid	15.2	Tyrosine	4.5
Proline	1.9	Phenylalanine	5.0

In our own experiments where activated sludge protein was used to replace 50% of the fish meal content of trout feed we observed no obvious pathological effects in the fish while the average body weight of the experimental fish was somewhat higher at the end of the experiment (72 days) than the controls (Table 7). Similar feeding trials in rats and poultry have demonstrated that it is possible to incorporate activated sludge protein into the diet without apparent toxic effects while maintaining high growth rates.

Table 7. Trout, mean body weight (g).

Days	Control	Experimental
0	69.6	71.4
20	92.9	86.8
45	100.0	111.4
72	123.0	127.1

These results illustrate the great potential value of effluents and surplus activated sludge as a source of protein for use in animal and fish feed.

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THE THERMOSTABLE CELLULASES OF MICROMYCETES AND THEIR APPLICATION IN THE UTILIZATION OF FOOD INDUSTRY WASTES.

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SUMMARY

The ability of more than 1000 representatives of different genus of micromycetes to synthesize extra-cellular cellulases in the range of temperatures 40-50° have been studied. It was shown that some selected thermo-tolerent isolates of microscopic fungi are capable of growing at the abovementioned temperatures.

A number of food industry wastes could be used as nutrient medium for the cultivation of thermo-tolerent micromycetes. In addition to protein rich biomass (up to 40%), cultural filtrates have high activities of cellulases. The properties of cellulases isolated from thermophilic and mesophilic cultures of micromycetes and perspectives of their application in the utilization of food industry wastes are discussed.

PROTEIN POTENTIAL FOR FOOD AND FEED RESIDUES OF ALCOHOL AND VEGETABLE OILS PRODUCTION IN THE BRAZILIAN ENERGY PROGRAM.

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SUMMARY

To increase the independence of Brazil in the energy field, a search for alternative measures for the production of fuels from renewable sources has been made. On this basis, alcohol and vegetable oils have been chosen as alternatives for gasoline and diesel oil, respectively.

By-products from alcohol production, using sugar and starch crops and ligno-cellulosic residues, that contain an appreciable quantity of protein or can be converted into a protein or NPN products, are alternative sources for food/feed production.

As possible sources in the production of protein concentrates, we consider cassava tops (approx. 30% PB), giant grasses (approx. 15%) and yeast produced in fermentation.

Stillage can be used as a liquid for SCP production or dried as a supplement for feed and cane sugar bagasse can be converted into a support for the better assimilation on urea by cattle, through the synthesis of carbamates.

Concerning the utilization of vegetable oils as diesel oil substitutes, the Vegetable Oil National Program foresees, in 1990, a production of 30 x 10^6 ton of residual cake. Besides the more traditional oil seeds, such as soybeans, peanuts and colza, incentives will be given for studies of native perennial species, due to their great potential in Brazil.

The resultant cakes from some of these native species, although they have a high PB content (approx. 60% for "*pinhao- manso*"), have toxic components, thus requiring research for detoxication by low cost methods.

This work intends to show the magnitude of non-conventional protein production potential from the Brazilian Energy Program and to discuss the main technological alternatives for its utilization as food and/or feed.

UTILIZATION OF CEREAL GRAIN MILLING BY-PRODUCTS AS FOOD RESOURCES.

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SUMMARY

This paper describes processes in commercial installations, and processes at the developmental stage in laboratories, which are used to recover protein concentrates and/or other nutrient concentrates from cereal grain milling by-products. Yield, composition, functional properties, and nutritional values are discussed for concentrates from corn, rice, wheat, oats, triticale, rye, barley and sorghum. The use of these concentrates in foods and feeds, and their potential worldwide is addressed. Recent successful work in the USA on stabilization of rice bran and the recovery of edible oil, together with the projected use of the stabilized bran as a foodstuff, are described in detail. The economics of these processes are also described in detail.