# 1766 BIOTECHNOLOGY IN DEVELOPING COUNTRIES **DELFT**, 1982 Editors: P. A. van Hemert H. L. M. Lelieveld J. W. M. la Rivière

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# BIOTECHNOLOGY IN DEVELOPING COUNTRIES





Proceedings of the symposium on Biotechnology in Developing Countries, held in Delft, The Netherlands, October 13-14, 1982.

Organized on behalf of:

- The Technical Microbiology Section of the Netherlands Society for Microbiology
- The Netherlands Biotechnological Society, Section of the Royal Netherlands Chemical Society.

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# BIOTECHNOLOGY IN DEVELOPING COUNTRIES

Editors: P. A. van Hemert H. L. M. Lelieveld J. W. M. la Rivière

Symposium, Delft, The Netherlands 13 and 14 October 1982



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#### FOREWORD

The organizers of the symposium are pleased to be in a position to publish the texts of the presented papers in response to numerous requests that were received before, during and after the meeting. Apparently the symposium fulfilled a need and it is our sincere hope that the interest it has generated - if only to a limited extent - will contribute to cooperation between developing and developed countries in the field of biotechnology. In this respect the participation of representatives of both the Commission of the European Communities and the Dutch Government was most encouraging.

The symposium - after a lively panel discussion - reached the following conclusions:

- Biotechnology may contribute considerably to the development of the Third World, in particular in the production of food, feed, fuel, microbial insecticides, in biological nitrogen fixation, preventive health care (vaccin production), waste treatment, crop improvement and microbial metal recovery.
- Developing countries can benefit from both small scale and large scale biotechnological processes.
- The lack of skilled staff, able to evaluate, select, and implement projects, is an important problem. Hence education in the fundamentals of biotechnology is essential. The Netherlands - in particular the Dutch Universities with a biotechnology programme - are able and prepared to assist in such education. Brain-drain (students not returning to their home country after having been trained abroad) might be kept to a minimum by applying the so-called Sandwichsystem of education: A great part of the Ph.D. study is done in The Netherlands, but the education is completed in the home country.

It is hoped that these conclusions will result in an increase in the number of students from developing countries participating in biotechnological courses in The Netherlands, with support from the government and/or the EEC. The organizing committee wishes to express its gratitude to the organisations that by their financial contributions made the symposium possible:

- Commission of the European Communities, Directorate-General for Science, Research and Development.
- Ministry of Foreign Affairs of The Netherlands, Directorate-General of Development Cooperation.
- Netherlands Biotechnological Society.
- Netherlands Society for Microbiology.
- Programme Committee for Biotechnology in The Netherlands.
  - Royal Institution of Engineers in The Netherlands, Division of Chemical Engineering.
  - Royal Netherlands Chemical Society.

Finally the Committee thanks the invited speakers for their contributions and for making their texts available for publication. These were reproduced largely as received with a minimum of general editing.

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Delft, November 1983

For the organizing committee: H.L.M. Lelieveld BIOTECHNOLOGY IN DEVELOPMENT COOPERATION: A DONOR COUNTRIES' VIEW

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#### ABSTRACT

Biotechnology is an area of great potential in development cooperation because (1) its diversity makes it suitable for both rural and industrial application, (2) "tropical" biotechnology is of great interest also to donor countries and (3) the developing countries are eager to apply biotechnology, and are preparing infrastructures capable of absorbing assistance.

Training in biotechnology is identified as the first priority. Furthermore, research and development as well as industrial and commercial undertakings offer mutually profitable opportunities for cooperation.

The paper discusses existing and new modalities for cooperation in these areas at the governmental, intergovernmental (UN) and non-governmental level.

#### INTRODUCTION

In the industrialized parts of the world, biotechnology, along with microelectronics and informatics is seen as a highly promising instrument for boosting national economies in the coming decades. The governments of the USA, Canada, UK, France, the FRG, the USSR, Japan, the European Community and also the Netherlands are all investing large sums of money in training, research and development in biotechnology in a time when scientific training and research as a whole suffer in most countries financial cutbacks.

All of these countries are also donors in development cooperation, hence it is logical to ask whether or not biotechnology must have a place in development programmes, and, if so, how this could and should be realized. This paper addresses these two questions from the donor countries' point of view.

IS THERE A PLACE FOR BIOTECHNOLOGY IN DEVELOPMENT COOPERATION? There are three main factors to consider here:

 Biotechnology, pluriform as it is, <u>appeals strongly to the two</u> basic foundations of development policies of donor countries,

including the Netherlands. Its low-cost, small-scale rural applications have a direct positive effect on health, nutrition and environment of the poor, and thus fit into the so-called first (or ethical) track of Dutch development policy, which is the immediate improvement of the living conditions of the poorest population groups.

The second (or rational) track of the Dutch policy aims to improve, on the longer term, the self-reliance of the recipient countries through strengthening of infra-structures. Also here biotechnology is a powerful tool as its industrial applications can upgrade raw agricultural products into commodities of greater local and export value. Illustrative examples are presented in Table 1.

#### TABLE 1

Applications of biotechnology

High investment, large scale; for industrial development	Low investment, small scale; for rural development	
Antibiotics	Biogas and composting	
Enzymes	Nitrogen-fertilizer	
Bulk chemicals	Pest control (B. Thuringiensis)	
Fine chemicals	Mushrooms	
Single cell protein production	Indigenous fermented foods	
Alcoholic beverages, etc.	Algae for fodder etc.	

2. It is in the interest of the donor countries to assist developing countries in building up their own biotechnology. This is not only because of the general argumentation that successfull development creates new markets for the West and promotes world stability by decreasing wide gaps in living standards. Biotechnology has a special additional feature in that it has the important role of optimising the usefulness of the worlds renewable resources produced in agriculture, and this must be done differently in different places, its applications being shaped by local climate, agricultural and socio-economic constraints and opportunities. Chad, China, Brasil, Indonesia and Mexico are examples of countries that have given very specific shapes to applications of biotechnology as imposed by local constraints (Table 2). TABLE 2

Examples of endogenous biotechnology in developing countries

Mexico	:	Pulque (Zymomonas)
China	:	Small scale anaerobic digestion (7 million units) use of <u>Azolla</u> for nitrogen-fixation
S.E. Asia	1:	Indigenous fermented foods (Tempeh, Ontjom, etc.) Tropical mushroom production Fishponds fed with wastes
Chad	:	Use of <u>Spirulina</u> algae as food
Brasil	:	Alcohol from cassava and sugar cane

Availability of substrates, prevailing temperatures, local tastes and traditions, market and labor conditions may render a process, unfeasible in one place, feasible somewhere else. Hence progress in biotechnology in the tropical regions will lead to an enrichment and diversification of biotechnology as a whole which the West could not achieve by itself. Just like in mining engineering and in agriculture, of which biotechnology is an extension, Research and Development must have a strong international dimension. Thus development assistance can grow out into North/South partnerships that are based upon division of tasks and mutual benefits.

Admittedly, North/South cooperation may turn some developing countries into potential competitors of the donor countries. However, in view of the diversity mentioned above, it is likely that ultimately complementation rather than duplication will emerge, thus minimizing competition and restricting it to the beneficial area of competitive innovation, again to the benefit of all.

Moreover, the West has little choice: The donor countries who cooperate in this process will benefit on the longer term, while non-cooperation, perhaps of some temporary immediate advantage, would lead to destructive competition in the long run. In the present economic crisis this factor of mutual interest gains in weight as some donors are reducing their foreign aid budgets. At the same time other donors, e.g. the Netherlands, wish to give more prominence to positive interaction between their own economies and development cooperation. With some countries, especially the NIC's (Newly Industrialized Countries, like Brasil, Singapore, Malaysia, S. Korea, Taiwan, etc.)

relations have become already more businesslike and approach normal economic and commercial relationships.

Agriculture and horticulture are probably the strongest sectors in Dutch industrial and economic innovation and both have strong international dimensions. It seems to me imperative that biotechnology, as an extension of these sectors, from the beginning follows this example.

3. Perhaps the most compelling reason for giving biotechnology a prominent place in development cooperation is that the developing countries themselves insist on it. This was evident at the United Nations Conference on Science and Technology for Development (UNCSTD, Vienna, 1979) and many regional conferences of Ministers of Science and Technology (CASTARAB, CASTASIA, CAST-AFRICA etc.) have recognized biotechnology as a high priority area in development. So has the Unesco and the Commission of the European Communities.

One of the main assets of many developing countries is their potential for producing renewable resources in the form of biomass and this can serve not only as food and fodder but also as fuel and as a source for agro-based chemical industry. Biotechnology is also a powerful tool in waste recycling, preventive health care, pest control and other environmental areas. It is not unfamiliar to many developing countries as many have already practised biotechnology in some form or another as mentioned earlier. Many of its applications are "soft" technology, requiring overall investments.

The rich renewable resources can only be exploited optimally by an adequate input of biotechnological knowledge and this is presently lacking in most developing countries. As a result the annual biomass production is now used for domestic food supply and also exported in the form of cash crop products to obtain hard currency for importing commodities, an enterprise subject to the economic hazards of capricious market mechanisms.

Undoubtedly, the developing countries will make strong efforts to make the most of their own resources by acquiring the science and technology necessary to do so. They will have learned the lesson from OPEC's history and would not be willing to export bulk raw substrates in the form of starch and sugars to serve the Western biotechnological industry, only to import back again its products. Thus it is likely that developing countries will exploit their own biomass and will direct their agriculture accordingly. Consequently, they are seeking continued and stepped-up assistance from the donor countries because they are determined not to miss this particular boat, fully aware as they are of the benefits to be gained. In creating this awaremess much has been done by the so-called Global Impacts of Applied Microbiology (GIAM) Conferences which started in the sixties and brought together decisionmakers and microbiologists from developing regions with scientists from the West to discuss regional problems in applied microbiology.

#### TABLE 3

Conferences on Global Impacts of Applied Microbiology (GIAM)

967
969
973
977
980

This existing awareness was confirmed by the strong positive response received by a recent travelling mission of the UN Industrial Development Organization (UNIDO) which assessed the interest of developing countries in setting up a new major international research centre for biotechnology and genetic engineering (ref.1). Hence we can conclude that the developing countries not only wish assistance in acquiring biotechnology, they are also ready to receive it, in fact, are already starting out on their own. India (ref.2) and Mexico have, for instance, set up a board for Research and Development in Biotechnology; Kuwait (ref.3) has a biotechnology division at its Institute of Scientific Research (KISR).

In summary, it appears to me that biotechnology is an important field for constructive activity in development cooperation with great promise for the future because:

- It meets the criteria set for the two "tracks" of development cooperation policies.
- It lends itself to mutual interest undertakings; development of "tropical biotechnology" is in the interest of donor and receiving countries.
- The developing countries themselves want it and infra-structures capable of absorbing assistance are already emerging.

HOW CAN DEVELOPMENT COOPERATION IN BIOTECHNOLOGY BEST BE IMPLEMENTED?

In most developing countries infra-structures for training and research are already emerging but this process is far from uniform. Growing tips in applied microbiology are found in university departments of agriculture, of biology or of chemical engineering, and very often medical microbiology is the origin of activities in general and applied microbiology. In many countries no critical mass of scientists and engineers has been attained and there the field is slowly being advanced by a few devoted, hard-struggling individuals. Government funding is often extremely limited and usually accompanied by too many demands for short-term, directly applicable results. Nevertheless in many countries, over the past 8 years or so, national societies of microbiology as well as regional federations of societies have been founded. Through meetings, newsletters, journals and contacts with international professional organisations they stimulate the formation of a critical mass of biotechnologists (Table 4).

#### TABLE 4

National societies for microbiology in developing countries (ref.4) (membership between brackest)

Asia (2404)	Latin America (1950)	Africa (500)
Bangladesh India Indonesia Iran Korea Phillipines Singapore	Argentina Brazil Chile Cuba Mexico Peru Venezuela	Algeria Egypt Morocco Nigeria
Thailand Turkey		

The Latin American Association of Biotechnology and Bioengineering (Alabib) was founded in 1978.

Compare: USA (22000); USSR (12000); Japan (5000); UK (3600); Netherlands (900).

Superimposed upon these "endogenous" local infrastructures we find the kaleidoscopic array of foreign aid from multilateral and bilateral sources, from private foundations and from industries, each with their own terms of reference and criteria, and usually dosed in short bursts of projects.

The success of these projects - and especially their continuation after withdrawal of the donor - very often hinges on the availability of local competent scientists and engineers who participate in the projects and are expected to carry on after their termination; these are called "counterparts" in the jargon of development cooperation. Too often projects are theoretically focussed upon some in itself laudable development goal while the availability of counterparts is taken for granted in analogy to the Western approach of setting a goal and then finding the people to help attaining it. In developed countries this usually is not a problem. In developing countries, however, the situation is the other way around: Abundance of worthy goals, scarcity of competent people. These considerations lead to the two general conclusions for the field of biotechnology:

- Increasing the number of competent biotechnologists by training should receive high priority.
- As long as local expertise is scarce, projects should be designed in response to realistic requests of competent individuals or institutes that are active in developing biotechnology rather than be designed to meet an armchair-conceived, abstract development goal.

Against this background I now propose to discuss development cooperation modalities in the three areas of training, of research and development and of industrial and commerical cooperation, respectively, paying special attention to opportunities for the Dutch biotechnological community.

#### Training of biotechnologists

The aim of assistance in this area is, of course, to help create adequate capabilities for training, research and development in the developing countries themselves, embedded in the local system of universities and research institutes so as to provide a genuine potential for independent, creative and innovative Research and Development. This can be done along two parallel lines: 1) By training of selected individuals in a developed country through fellowship programmes and 2) By direct assistance to strengthen training and research facilities in the developing countires, on a national basis or, where infra-structures are weak, first on a regional basis by setting up or strengthening regional centres. In both cases the training should be in the first place on the graduate and postgraduate level. Of course, the lower echelons of technicians are also very important, but their training can best be effected locally by local biotechnologists who after they themselves have been trained, should see training of their own supporting staff as a first task.

The development policy of some countries, e.g. Sweden and the Netherlands has a preference for concentrating training in the developing countries rather than having fellows trained in donor countries, which might lead to braindrain. Still I think it is vitally important to have, especially postgraduate training, in the industrialized world as a complement to local training in the developing countries. In the first place because this is the only quick way to acquaint the trainee throroughly with the field he is entering and in the second place because it is essential for his future work to have established direct contact with the world's scientific market place in biotechnology, which for the time being is located mainly in the West. As to the braindrain risk, this can be easily overcome by keeping the training course short (about one year) and by careful selection of participants, a method already successfully employed by the International Education system in the Netherlands for more than 25 years.

There are already quite a few examples of international training programmes operating on the two parallel approaches mentioned above (ref.5).

For the present discussion I must single out two specific examples (Table 5). In 1978 Japan set up, with the assistance of Unesco, the International Center of Cooperative R and D in Microbial Engineering at Osaka, with Prof. Taguchi as director. There micro-

biologists from S.E. Asia are trained for one year. They spend some 2 months together in Osaka for basic training and are then planted out for some 8 months to various universities to join local research tems. Finally, they come together again in Osaka for writing their reports and presenting seminars. Starting next year, Professor Bull and colleagues will start an international training course in the U.K. on exactly the same lines, participants to be recruited mainly from Latin America, Africa and the Arab states (Table 5).

#### TABLE 5

One year postgraduate course in biotechnology (Osaka, London)

L monorio	9 months	1 month
Training at central institute	Individual research at universities	For reporting, examination and evaluation at central institute

I think we can draw two lessons from these examples with respect to Dutch contributions towards training biotechnologists for developing countries:

- There is ample room for constructive contributions towards existing biotechnology training programmes in developing countries and little need for setting up new ones. Dutch development funds for fostering biotechnology could be efficiently spent through financial assistance in the form of "funds in trust" to UN programmes and through help in kind by providing experts needed in such programmes.
- 2) In view of the vast training needs there is ample room for following the examples set by Japan and the UK by setting up an International Training Course in Biotechnoloty also in the Netherlands, e.g. along the lines set out in Table 6. Of course, we should specialize in area's where the Netherlands have something to offer and also not refrain from enlisting the collaboration of universities in nearby countries like Belgium and the FRG. Besides the direct benefits for the development goals it is only realistic to point out that the Dutch efforts in biotechnology research would be strongly enriched by the participation of Third World scientists and that also valuable contacts would be established for future cooperation after the participants have returned to their home countries.

TABLE 6

Outline for postgraduate international course in biotechnology in the Netherlands

Duration: 12 months

Language: English

Organisation: existing international institute(s) in collaboration with universities, research institutes and industries

Subjects offered:

Fermentation engineering Mushroom production Enzyme engineering Waste utilisation - biogas production - composting Biological nitrogen fixation

Biological pest control Vaccin production Sulphur removal from coal etc.

Fellowships: Dutch government, UN organisations, European Development Fund, Industry. Participants to be recruited and selected in consultation with ongoing international programmes so as to reinforce these.

On a more individual basis the so-called "Sandwich Model" for Ph.D. study (Table 7) recommends itself. This model is also very valuable when applied in reverse, so to speak: a Dutch student obtains a Ph.D. degree at a Dutch University on the basis of work largely done in a developing country. A good example is the thesis by Nout (1982) on fermented beverages in Kenya (ref.6).

#### TABLE 7

"Sandwich" Model for Ph.D. study

Initiation of research Research work in Ph.D. obtained in home country e.g. the Netherlands in home country

#### Research and development cooperation

Although R and D cooperation activities to some extent overlap with advanced training, it is possible and useful to distinguish the following three categories of R and D cooperation in order of increasing involvement of the donor: (1) Stimulation of endogenous R and D; (2) Joint R and D and (3) R and D by the donor alone.

#### (1) Stimulation of endogenous R and D

These activities consist of providing tools, and leave the initiative and the responsability to the third world biotechnologist.

For this very reason I think this type of assistance is perhaps the most important one. It can be provided in many different ways:

- Providing access to microbial cultures available throughout the world and assistance in setting up national and regional culture collections. An example is the work of the World Federation for Culture Collections (WFCC) and the World Data Centre (WDC) of the Microbiological Resources Centres (MIRCEN) Network who publish a World Directory of Culture Collections and provide training for culture collection curators.
- Providing access to the literature and other biotechnological information by strengthening libraries by donated subscriptions and by arranging for computer links with the literature data . banks, which fortunately have the tendency of becoming cheaper and cheaper. Also special publications directed at biotechnological problems in developing countries can be very helpful; a beautiful example is the series published by the US Academy of Sciences which outline problem areas, provide selected references and also addresses of research contacts (ref.7,8).
- Tailor-made small grants to young researchers for equipment and travel as very successfully practised by the International Foundation for Science of which the Netherlands is a member through our Academy of Sciences.
- Measures to reduce the isolation of biotechnologists in developing countries, e.g. by enabling them to participate in congresses, symposia and workshops, which in their programmes then also must devote attention to problems of developing countries. This is now being done more and more in the Internationa Congresses of Microbiology of International Union of Microbiological Societies (IUMS) and the International Fermentation Symposia with the aid of UN organisations. A new tool of increasing importance for making a true world community of biotechnologists will be provided by computer communications by satellite for socalled computer conferencing and consultation, enabling institutes all over the world to have dialogues resulting in transfer of requested information. A first experiment will be undertaken by the International Development Research Centre (IDRC) of Canada and the World Academy of Art and Science (WAAS) with assistance of the MIRCEN network, institutes in Ottawa, Stockholm and Singapore participating.

These examples of stimulative assistance meet two kinds of problems: <u>First</u> the tendency of commercialisation and protection of information and also of special microbial cultures. In this regard the UN, i.a. through the World Intellectual Property Organization (WIPO), has a watchdog function, but it appears certain to me that we have to accept as a fact of life a situation, in which some knowledge is a commercial commodity. This means that, when necessary, knowledge will have to be bought with development funds. But let us not forget that most of the relevant information, fortunately, is free.

The <u>second</u> problem is that many, especially bilateral development programmes, are project-oriented and not easily accomodate such stimulative measures, not in the least because of the gap between the scientists and the government agencies for development cooperation. The fact that this symposium is supported by the Dutch minister for Development Cooperation means that initiatives of scientific societies are encouraged and I believe that such societies can play a significant role. The American Society of Microbiologists (ASM) has a special committee for development cooperation and the Americal Association for the Advancement of Science (AAAS) has set up a world wide organisation of scientific societies to assist sister societies in the third world, in which the Dutch Chemical Society participates (ref.9). I think also our societies could make a start in this direction (Table 8).

#### TABLE 8

#### Tasks for Microbiological Societies and Federations

- Draw attention to new developments and research problems in third world in special sections of journals and newsletters
- Publish cheap monographs on processes useful to developing countries
- Assist the Dutch government in biotechnology projects
- Increase participation of third world biotechnologists in meetings
- Stimulate visits of members to research centres in third world (lecture or study visits; sabbaticals; practical work of students).

#### (2) Joint R and D activities

While the previous approach is directed towards helping the third world biotechnologists to do his own R and D better and faster,

this approach has the dual aim of getting a specific result and in doing so of improving local R and D capability. An additional important advantage of local and foreign experts working side by side is that the results obtained are likely to be of greater and more lasting value. In my view it is of great importance that the drive and leadership in such projects should come mainly from the local scientists, and that the experts from abroad should have a passive, support role so as to avoid that, in their eagerness to achieve results, they create a solution that cannot work under local conditions without continued foreign support.

There are many area's in which the Dutch can offer R and D expertise (Table 9) and, again, I would like to emphasize that such activities can also be seen, at least in part, as long term investments, that may yield experience with tropical substrates and conditions as well as future orders for commercial and industrial enterprise.

#### TABLE 9

#### Area's for R and D cooperation

- Anaerobic digestion
- Biological nitrogen and phosphorus removal from waste water
- Composting
- Mushroom production
- Biological nitrogen fixation
- Dairy and other food biotechnology

- Biological pest control
- Vaccin production (human and animal)
- Enzyme production (e.g. isomerases, proteinases, urease)
- Fermentation (alcohol, antibiotics, gluconic acid, phenylglycine)

#### (3) R and D by the donor alone

It has been argued for a long time, and rightly so, that research activities, e.g. at Western universities should not restrict themselves in a provincial manner to problems of the country but also address problems of wider scope, including problems of the Third World, which as we all know is rapidly establishing itself as the biggest one. Obviously there are world problems of great significance which merit the best research efforts available to achieve quick results, such as new nitrogen-fixing crops or a remedy against schistosomiasis of which more than 200 million people are suffering. Such projects merit in my view a priority

in funding and Western cooperation comparable to that for "big" science areas like nuclear physics (Centre Européenne pour la Recherche Nucleaire (CERN)) and molecular biology (European Molecular Biology Organisation (EMBO). In this respect we can point to the success story of the eradication of smallpox as a historic example. The various UN agencies have shopping lists of research problems from which many topics for such projects can be drawn. In the Netherlands, the RAWOO (Advisory Council for Scientific Research in Development Problems) has the difficult task of stimulating and coordinating so-called "development oriented" research. This task is complicated by the fact that all sciences are involved and that coordination by selective project approval is hampered by scientists just giving a "developmental" twist to their project in order to get the funds. Moreover, there is the regrettable tendency to consider research for development as second rate research reserved for highly idealistic but second rate scientists.

These problems might, in my view, be overcome by setting up a small, rather autonomous, flexible agency manned by scientists of the most important disciplines in analogy with the International Development Research Centre (IDRC) in Canada, with the mandate to utilize, within its budget, the research potential of the Netherlands for the solution of important development problems to be recommended by an international Advisory Board. In addition, the system of tenders for research contracts should be mentioned in which universities, governmental and industrial research laboratories could compete and also collaborate.

The foregoing leads me to the following conclusions with regard to R and D cooperation:

- The Netherlands can and should participate in stimulating endogenous development of biotechnological infrastructures through international organisations as well as its scientific societies.
- In joint R and D activities care must be taken to leave the responsability as much as possible to the partners in the Third World. The Netherlands has a great deal to offer and to gain in various areas of biotechnological R and D.

- For development-oriented research in the Netherlands a special agency should be set up on the lines of the Canadian International Development Research Centre (IDRC). The use of the tender system for contract research is advocated.

#### Industrial and commercial cooperation

In the first place, industrial corporations from the West can be tempted to locate factories or research institutes in developing countries. Although this may have an important effect on development, I consider this aspect beyond the scope of this paper except for one comment: It appears to me to the advantage of both partners if such settlements do not remain foreign "black boxes" in the structure of the host country. Again through training and wise personnel policies such ventures will gain in stability and usefulness when they become enmeshed in the host country's own biotechnology infra-structure and are used to improve that. Secondly, nobody likes to invest time and money in reinventing the wheel. Similarly developing countries are not willing to follow, even if they could, at a high pace all consecutive stages of the evolution of Western industry. Where they can, they wish to profit from the so-called leap-frogging process and to start out with the best proven, and most modern industrial processes that suit their particular conditions. That means that they are inclined to buy licenses, patents and know-how, and when local expertise is lacking also consultancy services to assist in feasibility studies, negotiations and the final decision to buy or not to buy. Some countries, like OPEC countries can spend their own funds, others are in a position to use development funds, the donor in such cases providing consultancy services and terms of reference aiming to ensure that a wise decision is made. With regard to patents, there is a great pressure from the developing countries in various UN fora to remove this form of restriction of access to technological knowledge as much as possible. It is not likely that the industrialized countries will yield in this debate and it is likely that developing countries who produce their own patents will eventually change their views. Special pains must be taken to avoid that the buyer finds himself the owner of a process or an installation that does not work, as has happened frequently in the past. In the long run, as a result of training, developing countries will be capable of exercising

their own critical judgement but as long as this stage is not achieved every transaction should be accompanied by careful feasibility and adaption studies and also have a built-in training and back-stopping component to ensure that the process will keep running and that its installations will be properly maintained, serviced and repaired. In addition an environmental impact assessment should be made, whether or not demanded by law.

It appears that the Netherlands has a great deal to offer on this market. Table 10 shows areas in which this is the case (ref.10). For promoting activities in developing countries in these areas, interested parties might wish to consider setting up together a modest agency for market exploration and consultancy.

TABLE 10

Areas for industrial and commercial cooperation with the Netherlands; Processes, know-how, services, equipment

Anaerobic digestion	<ul> <li>upward flow sludge blanket</li> <li>fluidized bed</li> </ul>
en di ka yara yara ya	- two-phase process of the Institute for Pre- servation & Processing of Agricultural Crops
Waste treatment	<ul> <li>Oxidation ditches (Pasveer sloot)</li> <li>Adapted turn-key installations</li> </ul>
Industrial fermentat	ion processes, including brewering
Dairy and food biote	chnology
Biotechnology of pla	nts (new races, virus-free plants; new methods of cultivation)
Toxicity testing of	new products (e.g. single cell protein pro- duction)
Extension services f	or introducing new practices
Sharing of monoclona	1 antibodies

In summary, for industrial and commercial cooperation I come to the following conclusions:

- Foreign industrial settlements in developing countries should seek to establish the fullest possible integration with the local biotechnological infrastructure.
- Although care should be taken that new ventures are thoroughly adapted to prevailing socio-economic conditions there shoud be no hesitation in utilizing modern sophisticated processes and buying patent rights when the situation warrants this.

- The Netherlands has a significant potential for exporting biotechnological know-how to the Third World and should set up a small organisation of interested parties to promote this export.

In closing, I wish to draw attention to Antoni van Leeuwenhoek who was born in Delft almost exactly 350 years ago. It took some 200 years before Pasteur could continue his work and start building up microbiology, thereby laying the foundations for modern biotechnology. But even now, the great benefits derived from the results of van Leeuwenhoek and Pasteur have not penetrated all corners of the world. I hope this symposium in its own modest way will help to distribute the applications of biotechnology all over the world somewhat more rapidly than in the years behind us.

#### REFERENCES

- 1 Hedén, C-G. The establishment of an international center for genetic engineering and biotechnology. In: "L'impact des Biotechnologies sur le Tiers Monde", colloque organisé dans le cadre FAST de la Commission des Communautés Européennes en cooperation avec le CNRS et l'Unseco. Paris, 1982.
- 2 Agarwal, A. India sets up biotechnology board. New Scientist, Vol. 93, p 213, 1982.
- 3 Anonymous, MIRCEN News vol.2, p 29, 1981.
- 4 Anonymous. In: International Union of Microbiological Societies News Letter no. 5, p 11, 1981.
- 5 DaSilva, E.J., This publication pp. 19-57.
- 6 Nout, M.J.R. Aspects of the manufacture and consumption of Kenyan traditional fermented beverages. Thesis. Wageningen, 1981.
- 7 Board on Science and Technology for International Development. "Microbial Processes: Promising Technologies for Developing Countries", National Academy Press, Washington, 1979.
- Countries", National Academy Press, Washington, 1979. 8 Board on Science and Technology for International Development. "Food, Fuel and Fertilizer from Organic Wastes". National Academy Press, Washington, 1981.
- Academy Press, Washington, 1981.
  9 Scientific and Engineering Societies in Development, a Newsletter published by the Office of International Science of the American Association for the Advancement of Science, Washington, 1982.
- 10 Innovatieprogramma Biotechnologie. Kader, hoofdlijnen, operationeel plan, Den Haag, 1982.

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BIOTECHNOLOGY IN DEVELOPMENT OF COOPERATION: A DEVELOPING COUNTRIES VIEW

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#### ABSTRACT

This contribution deals with the promise of biotechnology with special significance for the developing countries: Today, biotechnology, or more particularly the applications of microbiology, can make a contribution to the socio-cultural, economic and technological aspects of development in developing countries. In justify-ing the increasing attention being given to microbial biotechnology by a number of decision-making and governmental bodies in the developing countries, instances of how such investments in the industrialized societies have paid dividends, are described. For example, in 1981, about 50.000 tons of high-protein animal feed derived from a methanol-based process using Methylophilus methylotrophus, was made available to European farmers. With relation to food additives, the annual production of citric and glutamic acids has been estimated at 100 and 200 million kilograms respectively. In Japan, the production of microbially-derived products is valued at US \$ 18.2 billion per year. Annual production of Single-Cell-Protein (SCP) in the USSR has exceeded 1 million tons.

Biotechnology is an apt vehicle for development because it can help developing countries use their natural renewable biomass resources to meet many of their requirements in the food, fuel and fertili-zer sectors. For example, the success of the bio-gas programmes in China, India, the Republic of Korea and Tanzania has shown how microbial activity can be harnessed on a technological scale to electrify villages, conserve soil fertility, preserve food through fermentation and even produce feed for animals. Of course, development is meaningless without a vibrant infrastructure, trained manpower and judicious use of available natural resources. Traditional "formulae" have involved bilateral assistance for specific projects and volunteer non-governmental organizations engaged in the spread of "appropriate technology". To overcome several bottlenecks, such as the lack of scientific knowledge (which should be earned rather than be bought), the shortage of trained manpower and the unequal distribution of natural resources, that plague particularly developing countries, the design and implementation of the network approach has been described. Such regional networks, e.g. that of the Southeast Asian Network in Microbiology, and the Unesco-sponsored projects of the Microbiological Resources Centres (MICRENS) and of the activity in Africa and the Arab States, indicate that the developing countries will have to look more and more to themselves in using microbial technology for development. In addressing their own problems with locally-thought out solutions, as opposed to alternatives imported or bought from the technicallyadvanced countries, developing countries can exemplify South-South cooperation. Industrialized societies, for instance, can co-operate with developing countries in meeting a priority need by organizing longterm postgraduate courses which will provide postgraduate researchers from the developing countries with the necessary knowledge and techniques that can be adapted to local processes. An example of such a course is the International Postgraduate University Course in Japan sponsored by Unesco and the Government of Japan. Such a mechanism aids in the evolution of manpower expertise and excellence as well counteracting the growing problem of "braindrain".

The presentation also describes small-scale technologies such as biogas technology, <u>Rhizobium-inoculant production and ore-leaching</u> that can not only catalyse development but also address socio-cultural problems.

#### BIOTECHNOLOGY: DEFINITION AND SCOPE

Biotechnology is not new. Traditionally known as industrial microbiology, biotechnology as a term has been employed with different connotations by politicians, fund-seekers and project-planners. It describes an industry that predates written history. In general, biotechnology emphasizes the application of biological systems to the manufacturing and service industries. Some attempts deal with the integrated use of biochemistry, agricultural biotechnology and medical biotechnology and the like. Recently, a group of experts dealing with the "manpower and training implications of the biotechnology-based industries" was unanimous in admitting that the term biotechnologist (or bioengineer) does not, at the present time correspond to reality (ref.1). In perspective, these attempts indicate that biotechnology as a new discipline or science is in a state of ferment due to interests from twelve major fields: Microbial genetics, industrial microbiology, zoology, cell biology, chemical engineering, biophysics, agronomy, ecology, botany, microbial physiology, computer technology and neurobiology.

Since this "new" science is actually 98 per cent applied and/or industrial microbiology, it is referred to as microbial technology which is complemented by other bio-processes that utilize biological species of non-microbial origin. Leaving aside the glamour of the catchword bictechnology, attention must be given to those microbial technologies (Table 1) that provide for:

Area of microbial activity	Products (examples)	Application in industry
Production of fermented foods	Natto, cheese	Fermented food industrie
Production and utilization of microbial cells	Single-cell-protein, food and fodder yeasts vaccines, bioinsecticides	Food. pharmaceutical and medical industries
Brewing	Wine and spirits	Brewing industry
Production of industrial solvents	Ethylalcohol	Chemical industry
Production of organic acidulants	Citric and lactic acid	Food and chemical industries
Production of macromolecular polysaccharides	Dextrans, xanthans	Food and allied industries
Production of antibiotics	Penicillin, Actinomycins	Medical and pharmaceutical industries
Production of physiologically-active substances	Vitamin B <sub>2</sub> , Auxins, Steroids	Medical, pharmaceutical and agricultural industries
Production of aminoacids	Glutamic acid, lysine	Food and feed industries
Production of mononucleotides and related compounds	ATP, 5'-Inosinic acid	Food and medical industries
Production of enzymes	Amylase, Protease	Relevant industry
Conservation of natural environment	Treatment of sewage recycling of waste waters	Relevant industry
Others	Leaching of ores, Biofertilisers	Mining industry,agricultural and other relevant industries

## Table 1 - Application of microbial processes to industry

Source: Unesco, 1975

- a. environmental management through the bioconversion of domestic wastes into non-polluting fuels such as methane, ethanol and methanol
- b. enhancement of soil fertility and stability through the direct application of sludge material
- c. wastewater treatment through microbial systems
- d. strengthening of public health programmes by elimination of enteric parasites through the anaerobic digestion process
- e. bioconversion of agro-industrial residues into valuable secondary products
- f. concentration and leaching of valuable minerals from low-grade ores
- g. substituion of toxic chemical products and pesticides by microbial preparations.

Reliance on microbial technology is historic, realistic and sound (Table 2). In the industrialized societies, the vast potential of microbial technology has catalyzed economic progress (ref.2).

#### TABLE 2

Global quantity and value of some microbial products

Product	Yield per annum	Value in 10 <sup>6</sup> US \$
Beer	$55 \times 10^6 \text{ m}_3^3$	22 500
Wine and other spirits	30 x 10° m <sup>3</sup>	15 000
Baker's yeast	600 000 tons	250
Feed yeasts	800 000 tons	400
Citric acid	290 000 tons	335
Antibiotics	8 000 tons	1 500
Glutamic acid	100 000 tons	300
Corticosteroids		325

Source: Kurylowicz, W. Microorganisms and their role in fermentation processes including biosynthesis of antibiotics. UNIDO document ID/WG.300/2, 1979.

The late David Perlman - the doyen of fermentation technology in the sixties - observed that on a global scale 145 companies used fermentation processes (ref.3) and that more than 85 of these ventures were engaged in the manufacture of food and feed yeast. Furthermore, located in some 20 countries, there were a total of

24 plants involved in the production of citric acid. Still again, of 179 products produced by microorganisms on a commercial scale to date, 91 are antibiotics, 27 enzymes, 10 organic acids, 2 solvents, 9 vitamins, 21 amino acids. Some of these compounds are manufactured in large amounts. E.g. the annual production of citric acid is about 100 million kilograms and of the food flavour adjunct monosodium glutamate about 200 million kilograms. According to Japan's Ministry of International Trade and Industry, the production of microbial products in Japan is valued at US \$18.2 x 10<sup>9</sup>/ year.

In the fifties, the commercial value of enzymes was estimated at less than US  $5 \times 10^6$ /year. In the sixties, the use of bacterial proteases as adjuncts to laundry detergents boosted the value of microbial enzymes to about US \$150 x  $10^6$ /years

The importance of antibiotics has been established by their deployment as curative agents, as research targets, as genetic markers and as aids in defining biochemical pathways. In the field of ecology, they are of significance as microorganisms are known to benefit directly in their ecological niches from the antibiotics they produce (ref.4). Several developing countries produce antibiotics that help conserve and yield a more healthy and productive population which can effectively contribute to the national economy and in this sense are an important index of economic progress (Fig. 1). In 1979, Japanese laboratories accounted for 7 of the 11 new antibiotics introduced across the globe. And so, it would seem that the value of microbiological products is rapidly gaining on national and international markets. In 1976, the world fermenter capacity devoted to antibiotics was estimated at 1.75 x 10<sup>6</sup> m<sup>3</sup>. The world antibiotics market with major sales of cephalosporins (15%), tetracyclines (18%) and penicillins (17%) was estimated at £4.5 x  $10^9$  in 1980. Total world market sales ranged between 15 and 20 per cent. In Japan, the combined usage of antibiotics in tonnage of blasticidin (1318 t), polyoxins (747 t), kasugamycin (7030 t) and validamycin (3515 t) was estimated at 12 615 tons.

In the USSR, some 90 production plants use bacteria on substrates like hydrocarbons, agricultural industrial and forestry residues.



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The extensive recycling of wood pulp, effluent from paper mills, corn cob waste, bagasse and animal waste not only aids environmental management and pollution control, but also keeps the process costs economical. Annual SCP production in the USSR, under the aegis of the All Union Hydrolysis Agency has exceeded 10<sup>6</sup> tons. Furthermore, through the state agency, <u>Glavmikrobioprom</u>, feed yeasts, amino acids, vitamins, enzymes and antibiotics are produced.

According to a Delphi study (ref.5) research breakthroughs are expected in the eighties and the nineties. The current decade itself is expected to witness the development of new nitrogenfixing plants, single-cell-edible-protein (SCEP), petrochemical substitutes, new predator-resistant crop varieties and new therapeutic and immunogenic substances. Though in the past two decades expectations in the fields of medicine and agriculture have not been fully realized, advances have been made in industry e.g. in the development of new microbes in the extraction of valuable minerals from ores, for the production of better biologicals such as vaccines, insulin, growth hormone, and for the development of petrochemical substitues for lubricants and pesticides.

The importance of microbial technology for the future is emphasized by the award of Yen 35 x 10<sup>9</sup> from the Japanese Ministries of Industry and Finance to a consortium of five biotechnological companies and towards the recently established national research institute on applied microbiology and its allied biotechnological aspects. Other nations, developed and developing e.g. Brazil, India, Signapore, have embarked upon the biotechnological path in their quest for some solutions to their probems and needs (ref.6). Still, one cannot help suspecting that with the current trends in the increasing use of microbial genetic processes, the patenting of "tailored" microbial species and the growing prohibitive cost in the transfer of technology, the deployment of commercialized biotechnology will actually deepen rather that reduce the gap of development.

In this context, the developed and developing nations must part, and again integrate, in their routes on the biotechnological path (Fig. 2) because of the diverse nature of the problems that afflict both societies. Coping with lower economic growth means reduced





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energy consumption, less pollution, increased recycling, improved exploitation of wastes, responding effectively in terms of trade to the newly industrialized countries (NIC), and the creation of more job opportunities. These strategies are beset by the predicament of soaring prices in response to the aspirations and expectations as regards leisure, automation in daily existence, educational and retraining programmes and the conservation of health (ref.7). Consequently, the choice of technological investment will be different.

In adopting biotechnology as the rallying-point for several national economies and in utilizing the microbial labour force as the vehicle of transition and progress, the movement towards technological parity by the developing countries is further catalysed by the incorporation of renewable energy inputs, selfsustaining development projects and regional collaborative activities.

#### DEVELOPMENT

A large number of countries view the possession and availability of natural resources as the key to economic development, as the best insurance for the success of "start-up" development and as the source of wealth generation for capitalizing the improvements made in the industrial and agricultural sectors. The emphasis on natural resources and their possession, hitherto, has been intrinsically linked, in terms of development, to non-renewable mineral resources, such as oil, and the metalliferous deposits, e.g. those containing copper. Scant attention has been given to renewable resources such as biomass and agro-industrial resources, which, through microbial intervention, could yield a variety of market products. Daily news-items indicating that several members of the OPEC cartel are facing developmental problems despite the abundance of their non-renewable resources seem to make a similar point.

The aims of development are many (Fig. 3) and the most important appear to focus around strategies designed to: a. improve the quality of the life of the people b. promote utilization of renewable resources c. facilitate the evolution of relevant technologies that must


Fig. 3. Organogram showing inputs and outputs in relation to research and training in science and technology for development.

serve as the base for industrial growth and employmentd. seed economic growth in urban and rural areas through just distribution of food, fuel and the like.

The developing world, in general, is comprised of some 122 countries that differ in degrees of industrialization. Even though the NIC countries such as Singapore, Brasil, India, the Republic of Korea and China, have shown the way, problems of increasing populations, different systems of culture, government and society and diverse economic approaches are significant factors. Moreover, each country interacts with different pressures from its regional and international environments (Fig. 4). Therefore, an approach in promoting development may be appropriate in one situation and unsuitable in another, either because it is too complex or insufficiently advanced.

Several pundits emphasize an improvement of the situation in the developing countries through the transfer of "appropriate technology" projects. Aimed at technology transfer, this naive and charitable approach is immediately bared in the discrepancy between the existing local infrastructural expertise and the transplanted large-scale technology which at first is a status-symbol and finally an unmanageable white elephant.

In an analysis of the prospects of the fermentation industries (ref.8) attention was drawn to the concrete role of the colleges and universities such as Iowa State, Cornell, Wisconsin and Massachussets Institute of Technology, and their contribution to fermentation development 1915 - 1940. Today, Japan is reaping the benefits of its investment in fermentation research which began effectively in the mid-fifties. The forecast, that young students from North America would go to Japan for training as a means to ensure the continuation of the fermentation industries in the USA appears to be coming true. In 1975, the Japanese Ministry of International Trade and Industries classified microbiology as a "priority technology" allocating 6 x 10<sup>9</sup> yen to around 200 projects. For the next ten years the sum allocated is 26 x 10<sup>9</sup> yen (ref.9). Some developing countries like Thailand and the Republic of Korea are now considering university-industry biotechnology programmes.



Fig. 4. Factors and influences governing regional and international development

In short, the development progress of centuries cannot be simply bought, generously and swiftly bridged through a "booster dose" of international financial largesse or through a "vitamin pill" of concentrated "know-how".Unfortunately, neither petro-dollars, calculated enthusiasm nor impatience can shorten the arduous task of upgrading inadequate educational infrasturctures to meet the demanding needs of expanding economies in the developing countries. Too often it is forgotten that intelligence and hard work are at the basis of development and not money.

It bears repetition that there is no single or multiple utopian approach in solving the problems of development which may be low Gross National Products per capita, a high birth rate, high unemployment, heavy dependence on agriculture-based economies, inequality of income, poor and inadequate health systems, low generation rates of capital and sagging infrastructures.

# INFRASTRUCTURE - PROBLEMS AND APPROACHES

The importance of creating a strong scientific and biotechnological base in terms of infrastructure and manpower to transform the latent potentials into realistic productivity in developing countries cannot be ignored. Most Arab countries continue to trade primary resources with the importation of technology (ref. 10). Development of endogenous technology and a societal appreciation of the impact of such technology on the national scene in these countries are limited. In order to offset the inadequacies in quantity, quality, the "brain drain" (Table 3) and the content and distribution of education in the existing educational infrastructure, an optimal strategy should focus on attracting expatriate know-how (Table 4) and excellence to teach in on-site training courses, on enticing, through appropriate job-compensation schemes, emigrant expertise to man technical and university educational schemes and "bonding" of fresh university graduates for service in the rural areas of the countries concerned. Scientific bottlenecks are many in the path of development. One of the key drawbacks is the lack and shortcoming of scientific data, which though on tap, are rarely used by policy and decision-makers. Inventories of potential microbial technologies, their proven pilot-studies, the relevance of such technologies to solving local

	Average number of immigrants per year in occupation						
Time Period	Professional, technical and equivalent	Engineers	Medical sciences	Scientists in natural and life sciences			
1962 - 1967	598	99	66	42			
1968 - 1971	1453	183	275	134			
1972 - 1976	1410	196	351	59			
1977	1805	251	527	41			
1962 - 1977	1141	160	236	. 70			
Total No.: 1962 - 1977	18255	2561	3777	1126			

Table 3 - Immigrants from the Arab world to the USA in the professional, technical and equivalent worker group, 1962 - 1976 32

Source: Department of Immigration and Naturalization, U.S. Govt.: Microfiche, 1970 - 1979

problems, their impact on the economy, the environment, society and the public sector at large, are often unavailable to decisionmakers in a digestible form.

#### TABLE 4

Expatriate manpower required by Saudi Arabia, Kuwait and Bahrain, 1975-1980

Occupation	Saudi Arabia <sup>*</sup> (%)	Kuwait <sup>XX</sup> (%)	Bahrain <sup>X</sup> (%)	***
Managers	6.1	0.7	1 (Carl 1977)	
Professionals	7.8	-	0.3	
Technicians	49.9	11.7	1.6	
Clerical Workers	90.4	4.8	_ 3.8	
Sales workers	65.5	1.5		
Service workers	98.1	40.3	- 5.5	
Operatives	26.3	18.6		
Skilled workers	54.8	9.3		
Smei-skilled workers	99.7	-		
Total	498.6	86.9	17.2	

The shortage of trained manpower and its endogenous development is another bottleneck. The institutional bias and basis in several developing countries, dependent on inadequate educational infrastructures, has been to use experts from the industrialized societies. Insufficient use is made of the many skilled people in their own developing countries, who are far better equipped with an understanding of the local conditions. An optimal solution would be to have a balance of local and foreign expertise teaming up without the constraints of hierarchial inequality. Due to a shortage of scientific and technical personnel, only about 2 per cent of all research and development in new technologies takes place in the developing world (ref.11).

In order that developing countries reach some sort of industrial parity with the industrialized societies, attention has been given to several vehicles of assistance such as bilateral and multilateral assistance and sound technology transfer. Technology transfer, broadly speaking, from North to South has been supportive in the realization of such goals and growth patterns common to the industrialized societies. Direct local participation, an important aspect of self-reliance and self-development, is minimal in the "social and technical processes of developing, implementing and regulating a technology".

The evolution of biotechnological growth in the developing countries is related to the environment, the resource availability and to national growth. It involves the will to explore the path of selfreliance through a commonality of efforts on a national level that gradually expresses itself in regional co-operation.

Within the framework of the Regional Project in Microbiology in Southeast Asia, attention is given to the strengthening of research and training, on national and regional scales. As a prerequisite, a strong national infrastructure had to be developed for the diffusion of knowledge within the country and from the regional to the national level. The infrastructural growth crystallized from the joint responsibility and dialogue between the respective governments and the national scientific communities into nodal points defined as national points of contact. The node is often an identified research institute. The national points of contact are the pillars of the network structure and serve as feeders to the bodies and organizations forming the national net. As a result of such involvement, professional microbiological societies have evolved in the Philippines, Indonesia, the Republic of Korea, Malaysia and Hong Kong.

Credibility of the national point of contact, i.e. the identified research institute, is determined by its status in the national and regional arenas, its role in manpower training and upgrading of local scientific knowledge and the catalytic ability to develop relevant research programmes that are in consonance with identified national and regional needs.

Infrastructural development and its fleshing results from active national points of contact undertaking surveys of microbiological activities, identification of research institutes, universities, a) Policy



b) Operational



- NPB - National Participating Bodies - National Point of Contact (this will normally also be an NPB) NPC
- HRN - Headquarters of Regional Network
- RCB Regional Coordinating Board

Fig. 5. Structure of the Network of the Regional Project in Microbiology in Southeast Asia.

industries and governmental bodies that are involved in microbial technological practices and policies, contribution of biotechnological inputs into national development plans, identification of priorities in research and manpower formation, provision of aid in the acquisition of equipment and the organization of appropriate fora for the presentation of research results. Another important function is the organization, publication and release of a periodical newsletter that provides information on biotechnological developments in each participating country of the network.

# MICROBIAL TECHNOLOGIES: THEIR PRIORITY AND SERVICE IN NEEDS OF DEVELOPING COUNTRIES

The world's fifth largest and seventh most populous country in 1980 imported about 85 per cent of its petroleum at an estimated value of US \$11 x 10<sup>9</sup> which in turn left its mark on the country's economic growth through rising inflation and a growing foreignexchange debt (ref.12). To offset this huge expense to the national exchequer, Brazil in 1975 turned to biotechnology for a solution which was formulated in a US \$5 x 10<sup>9</sup> National Alcohol Porgramme (NAP) which by the end of the century is expected to yield alcohol sufficient to account for 75 per cent of all liquid combustible fuel consumed. Several fora and publications have attested to the significant contribution of biotechnology to the economic and energy sectors of Brazil. However, a word of caution is necessary in order to temper enthusiasm with reality as in the case of Kenya's embarkation on a national alcohol programme which has now been shelved. Though the Brazilian programme is a success from a technological point of view, the socio-economic objectives of the programme are yet to be fully met. In basing the programme solely on the purposes of reinforcing the sugar agro-industry and its value as an energy feedstock, the problems of regional inequality, rural-urban migration of the labour force, rural unemployment and inadequate income distribution have grown with alcohol production. Enthusiastic land acquisition and concentration for sugarcane, babaçu, palm, sweet sorghum, eucalyptus, yams, sweet potatoes, bamboo, and the exotic possibilities such as marmeleiro (Croton sonderianus), aveloz (Euphorbia tiricalli) and tropical water lilies have led to food supply shortfalls and displacement of rural industries.

Alcohol production has been extensively used as a petroleum substitute prior to World War II in Chile, Cuba, Czechoslovakia, Denmark, Austria, France, Germany, Hungary, Italy, Yugoslavia, the USA, Sweden, Australia, Ecuador, Malaysia, Mauritius, Paraguay and India (Table 5). Nowadays, based on the success in Brazil which is the third largest alcohol producer after the USSR and the USA, several developing countries have embarked upon the use of alcohol as a petroleum alternative. Plans and projects exist in China, Kenya, Malawi, Guatemala, Libya, India, Pakistan, Thailand, Colombia, Paraguay and Costa Rica. In Mexico, the alcohol programme has been proposed as a solution to the severe air pollution in the city.

#### TABLE 5

Alcohol production - Past and present status\*

PRE-WORLD WAR II PERIOD (1920 - 1940)

Countries with local or national programmes:

Argentine, Austria, Australia, Brazil, Chile, Cuba, Czechoslovakia, Denmark, Ecuador, France, Germany, Hungary, India, Italy, Malaysia, Mauritius, Paraguay, Sweden, South Africa, USA, Yugoslavia

POST-WORLD WAR II (1974.....)

Developing countries:

Argentine, Brazil, Cameroon, China, Guatemala, India, Indonesia, Kenya, Libya, Malawi, Mexico, Pakistan, Philippines, Republic of Korea, Senegal, Sri Lanka, Sudan, Thailand, Zimbabwe

Developed countries:

Australia, Austria, Canada, Denmark, Finland, F.R.Germany, France, German Democratic Republic, Greece, Ireland, Italy, Japan, New Zealand, South Africa, Switzerland, U.K., USA, USSR, Yugoslavia

x = list not complete; based on literature survey

The "alcogas" programme, launched in 1980 as part of the National Fuel Alcohol Programme, in the Philippines, aims at the utilization of local vegetable wastes as a source of anhydrous alcohol. The attractive feature of the venture is based on biomass conversion

that does not divert sugar or starch crops away from potential food markets as is the case in Brazil which uses molasses and Mexico that deploys cassava stocks. In Indonesia, the national programma - started in 1978 as part of the National Policy Plan "Repelita III" and implemented through the Agency for Development and the Application of Technology - is aimed at assisting the development of the labour force in the transmigration programme of 500 000 families rather than for use in the reduction of the transport fuel requirements. With the aid of a US \$6 x 10<sup>6</sup> grant from Japan, a Biomass Energy Research and Development Centre is being constructed that includes a pilot plant for alcohol production at Tulang Bawang in the Lampung region. Other goals are to develop unused lands outside densely populated urban regions, increase the transmigrant/farmer standards of living and promote the development of industries ancillary to ethanol production.

Sewage systems have often been referred to as a barometer of a nation's culture. In a number of developing countries, a microbiological technology, often referred to as biogas technology, is being deployed, to some extent, to meet their fuel, feed and fertilizer requirements. This technology, often described as a technology for the developing countries, is a technology that is capable of providing energy, improving rural living conditions and, of conserving the environment (Table 6).

# TABLE 6

Chinese biogas programme - ten point benefits

1. DIGESLEIS SAVE LUSSII	5	SLEIS	save	IOSS.	L 1	Tue.	$\mathbf{LS}$
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- Requires small labour force
  Saves fuelwood and grasses
- 4. Saves straw and other crop residues for animal fodder and bedding
- 5. Reduces expenditure for fuel
- 6. Reduces household labour
- 7. Eliminates many insect pests and diseases and markedly improves hygienic conditions for rural areas 8. Protects forests and timber
- 9. Enables mechanization of some rural crop processing tasks and local electricity generation
- 10. Narrows the gap in the standard of living between cities and villages

This is a technology that extracts the energy content of human and animal wastes, that preserves the fertilizer value of these wastes and that helps conserve human health resources against contamination of water supplies by human waste and air pollution arising from the use of solid biomass fuels in the domestic sector (ref.13). Though successful, there are some drawbacks in the implementation of the biogas programmes, particularly in Papua New Guinea, Fiji, Thailand, India, Nepal and several other countries. The reasons for these drawbacks is that biogas technology as practised is "second-class technology" and lacks appropriateness which implies an intimate integration with daily rural life and a sociopsychological acceptance by a majority of the rural population. Furthermore, the technology has been promoted, to some extent, by the trendy and elite groups that have been caught up in their selfappointed roles as agents of charitable development.

Only in China "household-scale" biogas technology has become thoroughly integrated into the economic and social rural patterns of existence (Table 7). Biogas in China is used to drive internal combustion engines for transport, water-pumps for irrigation and grain processing, and rural electricity stations.

Despite these successes, the Chinese biogas programme is beginning to stall (ref.14). Only half of the total number of 7 million units are reported to be working.

Indian and Chinese biogas techniques have established the anaerobic digestion system as a valuable source of energy. Requiring a modest investment, the construction of the underground digesters does not involve costly construction and insulation materials. The residual sludge material has potent fertilizing value.

At he "community-agroindustrial-scale", biogas technology is in operation at Maya Farms in the Philippines. As from June 1981, an "integrated-livestock-meat processing and canning operation", covers 36 hectares of land in the Antipolo Hills. It possesses a population of 25 000 pigs, 70 heads of cattle and 10 000 ducks. Ten biogas plants producing 75 000 cu.ft. of biogas and 4 tons of feed material per day from the manure and the wastes (coming from

Province	Country	Commune	Substrate and Digester Size	Biogas Utilization	Benefits
Anhui	Fengyang		the transferring and	Flue- curing of to- bacco	Improved management of manpower resources
Guangdong	Foshan		Night-soil (28 digesters with total volume 1316 cu.m)	Generation of elec- tricity Rural elec- trification	Improved public sanita- tion Biogas sludge used as fertilizer material Generator linked with lo- cal power grid
		Leliu	Xinbu System	Integrated village technologies	Improved livelihood of 89 families
Henan	Nanyang	or 14 St V Basing	Distillers' grains (Two digesters with total volume 4000 cu.m)	Development of local industries	Extraction of vitamin B <sub>12</sub> from biogas effluent Production of di- and tri- chloromethane
Jiangsu	Wujin	Bennin	Digester size: 1200 cu.m.	Generation of elec- tricity for distill- ery and brick kiln	Improved rural domestic facilities
Shandong	Quindao		Night-soil (digester size 2040 cu. m)		Improved sanitation
Shanxi	Xi'an		Sewage effluents with di- gester size of 5400 cu. m.		Improved sanitation
Siuchan	Quxian	Jingbian	n de la constante de la constan En constante de la constante de	Development of dual- fuel engine for water pumping and irriga- tion of rice-fields	Improvement in agricul- tural practices
		Pingxi	Digester size 150 cu. m.	Drying and baking facilities	Development of local industries
Nanchong		Xiangyang	Mint 11, vatily of vit	Biogas runs dual-fuel engine in milling of wheat, dehusking of rice	Improvement of local technology
	Chengdu	Dragon pond	Night-soil	Routine use in disposal of wastes	Improved public hygiene
ine na i ne	Rongxian	enar ege to tak	Distillers' grains and wate liquors (digester size)	Biogas used for dri- ving of trucks and generation	Generator linked with state power grid; saving of diesel fuel; sludge used as fertilizer; coun- teraction of aquatic pollution
	Deyang		Cowdung (digester size 1680 cu.m)	Generation of elec- tricity for farm use and rural life trough the IBS	50 per cent self-suffi- ciency-achieved in pro- vision of over 200 homes
	Wenjiang		Night-soil	Routine use in dis- posal of wastes	Improved public hygiene
Carrieran Att. att	Ziyang			Flue-curing of tobac- co	Improved management of manpower resources; Development of indige- nous technology for dry- ing cereals and grains

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#### Table 7 - Some Benefits of Biogas Technology in China

the farmland) are in continuous operation. Two more biogas plants that yield 51 000 cu. ft. of biogas per day from the wastes of 17 000 pigs are under construction. The biofuel generated is used for cooking and heating in the canning plant and the canteen, fueling 16 internal combustion engines for pumping water supplying electricity to the dormitories, canteen, meat-processing plants, slurry to and from the digesters, and the powering of the feed mill.

In November 1981, the Ministry of Agriculture in India launched a US \$62 x 10<sup>6</sup> National Project for Biogas Development which aims at the establishment of 400 000 biogas plants during the Sixth Five-Year Plan. The project is aimed at rural development, provision of rural electrification, energy supplies and fertilizer production. The project will be integrated with <u>Operation Flood</u> which aims at covering 10 million families and rearing a national herd of 10.5 million cattle of improved variety. The project will be implemented through a number of "biogas cells", at the state level, which will operate through a cooperative infrastructure providing extension, training and mass communication facilities. The programme is expected to provide employment to over 2000 educated unemployed personnel and to some 40 000 skilled and unskilled workers.

Here, attention must be brought to the "Madras experiment" (ref.15) where self-reliant perspectives for poor rural communities have been widened. Farmers in the village of Injambakkam cultivate less than half of their available rice lands on account of the prohibitive costs of imported fertilizers, diesel for water pumps and kerosene for lighting. A programme using biogas plants, medicinal plants to replace imported insecticides, the establishment of nitrogen-fixing cyanobacteria in the fields and aquaculture, made the village not only self-sufficient but also an exporter of food and energy. Observers from the University of Strathclyde looking at these developments begun in 1980 by Dr. Seshadri, indicated that within a 12-year span, food consumption can be increased by 200 per cent, indigenous energy production by 85 per cent, and overall solar energy capture by 150 per cent (ref.15).

National programmes in biogas technology have been described in Indonesia, Samoa, Thailand, China, Vietnam, India, Sri Lanka, and the Republic of Korea. Whereas lack of public acceptance, plant management and sociological problems were identified as obstacles in Indonesia, Samoa, Thailand, Tonga, India and Sri Lanka, the lack of adequate financial assistance was a serious drawback in Papua New Guinea and Bangladesh. Biogas activities have been reported in Bhutan, Brunei, Singapore, Cook Islands, Pakistan and Afghanistan (Table 8).

#### TABLE 8

Biogas technology and its world-wide practice

Purposes: rural development, disposal and utilization of wastes, fertilizer production, environmental management, village electrification, domestic fuel production

Asia and the Pacific:

Afghanistan, Australia, Bangladesh, Bhutan, China, Fiji, India, Indonesia, Iran, Korea (Rep. of), Malaysia, Nepal, Pakistan, Papua New Guinea, New Zealand, Philippines, Singapore, South Pacific Islands<sup>\*</sup>, Sri Lanka, Thailand

Arab States: '

Algeria, Egypt

Africa:

Botswana, Cameroon, Congo, Ethiopia, Ghana, Kenya, Lesotho, Malawi, Mauritius, Negeria, Rwanda, Senegal, Sierra Leone, Tanzania, Upper Volta, Sudan, Zambia, Zaire

Europe and North America:

Belgium, Canada, Denmark, Fed.Rep. Germany, France, Greece, Ireland, Israel, Netherlands, Sweden, Switzerland, Turkey, U.K., U.S.A.

Central and South America:

Argentine, Barbados, Chile, Costa Rica, Colombia, Ecuador, El Salvador, Guatemala, Guyana, Honduras, Jamaica, Mexico, Nicaragua, Trinidad and Tobago

x = Cook, Solomon, Tokelau, Kiribati, Nauru, Niue, Tonga, Tuvalu, Vanatu, Western Samoa

Based on literature survey; not exhaustive

As long as fertilizer energy is easily acquired and available in the industrialized societies, the use of biofertilizers and bioinsecticides will be restricted. This is borne out by a report

(ref.16) which states that "meanwhile other research is being done in many places which might threaten the existence of the fertilizer and pesticide industries, namely research into biological fixation of nitrogen and biological control".

Food production per capita in several developing countries has been reported to exceed that of the developed countries (ref.17). The spread of high-yielding varieties of staple foods like rice, wheat and maize has reduced and sometimes eliminated the need for grain imports in some developing countries thus conserving capital for development (ref.18). The bridgeheads achieved are, however, often plagued by the menace of storage losses (Fig. 6), an everclimbing population growth index and the world food trade pattern. Choice produce and top-class commodity products, whilst catering to refined palates in the affluent societies, are often exported to increase local reserves of foreign-exchange.

Biological nitrogen-fixation (BNF) technology has been recommended for use in developing countries. Such a recommendation is sound, since chemically fixed nitrogen fertilizer is characterized by steep economics, potential environmental problems and prohibitive acquisition costs. The use of <u>Rhizobium</u> inoculant material has many advantages, viz:

- a. A saving in huge quantities of fossil-derived energy necessary for the production of fertilizer
- b. A reduction of water pollution resulting from fertilizer percolation into ground water reservoirs
- c. A reduction in the use of expensive specialized equipment to fertilize crops.

Optimum <u>Rhizobium</u> has been shown to provide up to 25 per cent of the total nitrogen required by the plant. Recently, a low-cost bio-technology, based on renewable natural biological algal resources has been developed (ref.19). Designed for rural environments, such technology, easily accessible and devoid of sophisticated operations, could provide 20 to 30 kg of biologically fixed nitrogen per hectare of rice crop per season (ref. 20). The algal culture is a soil-based mix of <u>Aulosira fertilissima</u> and several other filamentous species of the cyanobacteria. Experimental evidence has shown that application of such fertilizer can bring about a

Fig.6: Loss of Food Commodities during 12 month storage period in selected countries.



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10 - 15 per cent increase in crop yields, could save chemical nitrogen fertilizer to the extent of 20 to 30 kg N/ha without diminishing crop yields and soil fertility. An added advantage is that the technology encourages the farmers to produce their own requirements making redundant the establishment of high-cost centralized industries. Blue-green algal fertilizer technology recommends itself by virtue of its generating rural incomes, employment potential in rural development programmes and decentralized self-reliance. Such technology is widespread in India through the All-India Co-ordinated Project on Algae and has been introduced into Burma, Sri Lanka, Nepal and Bangladesh.

Other promising technologies for deployment in developing countries are those that involve microbes in the production of bioinsecticides and in ore-leaching.

Nicaragua, a decade ago, imported US \$10 x 10<sup>6</sup> worth of chemical pesticides for protection of a cotton crop worth US \$60 x 10<sup>6</sup>. The use of microbial agents for biological control, according to an FAO study, could reduce expenditures for pesticides by 50% (ref.21). Several types of bioinsecticides are known: Bacterial, fungal and viral (Table 9). Such type of insecticides may help stem the deep inroads being made into storage of edible commodities by pests and insects. Futhermore, they may help bypass the usual energyintensive, toxic, broad-spectrum chemical insecticide production approach adopted by the industrial nations. Though, they can be produced in bulk by fermentations (chiefly bacteria and fungi), in living insects (some bacteria, viruses and most protozoa), and in tissue culture (viruses), bioinsecticides apparently harm neither non-target organisms nor the environment. Vigilance, however, is still required to avert unforeseen catastrophes. Several developed and developing countries are engaged in mounting effective programmes involving the substitution of chemicallyderived products by microbial insecticides.

Recent research advances in using microbes for the successful extraction and recovery of metals indicate the advent and potential of a new microbial technology - biometallurgy or geomicrobiology which may provide a possible answer to the crisis looming on the 46

# Table 9 - Microbial species used as bioinsecticides

Group	Organism	Target	Field of Investigation	Collaborating Institutes
Bacteria	<u>Bacillus sphaericus</u> 1593	Blackfly larvae	Environmental safety	University of California (Fresno) Lee County Mosquito Con- trol District (Florida)
	B. <u>thuringiensis</u> var <u>israelensis</u>	Mosquito and Blackfly larvae	Environmental safety	Institut Pateur (Paris) ORSTOM (Bondy) Institute of Entomology (Prague)
Fungi	Coleomyces dodgei	Mosquito	Mass production	University of California
	<u>C</u> . <u>opifex</u>		Mass production; Mammal safety	University of Dunedin
	<u>Culicinomyces</u> <u>clavosporus</u>	Mosquito	Safety studies on target and non-target vertebrates and non-vertebrates	Royal Australian Medical Corps University of Syd- ney University of California
	Lagenidium giganteum	Mosquito	Safety studies on non-tar- get organisms	University of California USDA (Lake Charles Station
Protozoa	<u>Nosema</u> <u>algerae</u>	Mosquito	Safety studies in mammals	University of Texas USDA (Gainsvill Station) Pakistan Medical Research Centre
	<u>Vavraia</u> <u>Culicis</u>	Mosquito	Mass production	Czechoslovakia Academy of Sciences
Viruses	Baculoviruses	Crop pests and worms	Production of viral pesticides	Several countries engaged in R & D and marketing
Nematodes	Romanomerniis culicivorax	Mosquito: Anopheles Albimanus	Field studies in El Salvador Ivory Coast Western Samoan Islands	USDA (Lake Charles Station Newfoundland Memorial University and University of Otago

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horizons. The reporting of the hiking of prices of tin in Malaysia and the concerns of the copper-producing nations are serious indicators of the pressures on the availability of minerals. Like oil and food, the acquisition of strategic nonfuel minerals is being enmeshed into socio-economic strategies. One of the most industrialized countries is dependent upon 24 of the 32 strategic and critical minerals which originate in exports from Zaire, Zimbabwe and Zambia. Copper recovery from ores and ore wastes by natural microbial activity on a world-wide basis has been estimated at 300 000 tons per year (ref.22). At its plant in Totomocho, Peru, the Andean Development Coroporation of the Cartagena Accord is expected to obtain 25 000 tons of copper by microbial action. The technology of biomining is expected to develop rapidly as the energy costs rise and as the supply of high-grade ores become limited and less accessible (ref.23).

# ASSISTANCE FROM DONOR COUNTRIES

The provision of assistance from donor countries towards meeting the needs of the developing countries is governed generally by four criteria:

- a. The project should address the technical social and economic needs of the recipient country
- b. The project should involve shared responsibilities by both partners in the development and management of the project when and where necessary
- c. The project should be aimed at the promotion of self-reliance in the developing countries
- d. The final result of the project should be of benefit to both partners

Developing countries in need of assistance are often faced by two major problems, viz. the development of endogenous scientific and technical expertise and the need to be in the mainstream of new scientific advances. By experience, most developing countries can benefit most by undertaking applied research programmes that on the one hand address basic research activities and on the other hand permit them to experiment with innovative breakthroughs under local conditions. Assistance towards programmes between the developed and developing countries may be either bilateral, multilateral, regional or international in character (Table 10). Bilateral or multilateral assistance involves a sustained long-term dialogue and partnership between selected research institutes in the participating countries. Assistance is aimed at developing a self-sustaining capacity in the recipient developing country and often assumes the availability of sufficient manpower and technical facilities for the joint implementation of the project. The main features of such assistance are: Joint study projects, exchange of staff, information and training. The issue of joint management is often neglected or in most cases residues with the donor country. Regional assistance programmes may be of two types i.e.:

- a. assistance provided by a donor country to a group of regional countries via an intermediary such as a UN-agency like UNESCO, and
- b. the pooling together of resources by the developing countries towards a regional centre that is created and managed by a group of developing countries in a particular region.

The first scenario is often referred to as Funds-in-Trust project; the best example is the Southeast Asian Microbiology project. In brief, since 1974 the Government of Japan, has made available an annual magnanimous grant to Unesco which is specifically designed for the promotion of training and scientific research through collaborative projects, fellowships and specialized symposia for the participating countries of the region viz: Malaysia, Philippines, Thailand, the Republic of Korea, Indonesia, Singapore and Hong Kong. In addition Australia and New Zealand are active participants and on ad hoc basis provide support. In the second scenario, the trigger for regional co-operation is a move towards political co-operation such as the East African Community in East Africa, the Andean Pact in Latin America, and ASEAN in Southeast Asia. The regional centre helps increase the input of assistance offered to the region involved. In any case, the regional approach is useful to developing countries that possess limited resources and insufficient cadres of trained manpower.

International assistance involves the combined efforts of governments, non-governmental organizations or individuals. Apt examples

# Table 10 - Project Areas with bilateral and other assistance

Country	Donor Assistance	Project Area
Algeria	Japan, UNDP, UNU	Integrated village project at Bou Saada
Argentine	USA	National Compilation biomass resources
Argentine/ Colombia/ Costa Rica/ Guatemala	OLADE, OAS, MIRCEN	Biogas Deployment for electricity and fertilizer
Burundi/ Upper Volta/ Rwanda	EEC, Belgium	Biogas digester construction, Fuel production in Ciney Village and Lake Kivu
Cameroon	F.R.Germany	Biogas scheme for rural development
Cape Verde	USA	Rural development
Egypt	USA	Rural development technologies
Ethiopia	F.R.Germany	Fuel production
Guinea-Bissau	Netherlands/USA	Biogas production on Bdoma Island
Ivory Coast	EEC	Utilization of agricultural wastes
India/China	F.R.Germany	Information dissemination in biogas technology
Lesotho	UNDP, UNESCO	Combination solar energy and biogas use
Malawi	Sweden	Commercialization of biogas in Blantyre
Niger	USA	Rural development; use of mycorrhizae
Rwanda	USA	Promotion of Biogas technology
Senegal	UN Centre for Energy	Development of rural energy centre
Sudan	F.R.Germany EEC Philips-Holland	Biogas from water hyacinths Fuel production from molasses Biomass conversion into utility products
Tanzania	Sweden/UNIDO/ Netherlands	Biogas use in Kongwa, Chilomwa and Makangua villages with Sido and AATP
Upper Volta	EEC France	Alcohol production from molasses Microbial production of methanol at Ouagadougou and Saria
Zaire	Belgium	Promotion of biogas use and techniques

Based on survey of several documents and papers.

PROPERTY AND A DOCUMENT

are the international agricultural research centres, EMBO and the like.

"Consciousness of the total value of the co-operative programmes - whether bilateral, regional or multinational - would not only help developing countries strengthen their scientific-technological infrastructure; it would surely provide new incentives to industrialized countries to increase the proportion of their R & D budgets consecrated to the needs of the economically less advanced countries" (ref. 24, 25).

#### ROLE OF SOUTH-SOUTH CO-OPERATION AND OF REGIONAL NETWORKS

The rationale for a network, whether regional or international in character, arises basically from the need of different elements, be these individuals and/or institutes, agencies or countries, that agree to focus on complementary problems in their own nationally-administered projects and to share, without vested interests, expertise, experience and technical know-how. The network approach, additionally, helps to overcome the problem of unequal distribution of resources, skills and information that perpetuate inequality. Two different patterns of growth are characteristic of networks and their organization (ref.26) viz:

- a. Where development time is necessarily long as a result of harmonizing different inputs from network participant components, and,
- b. Where development is necessarily short due to the inadequacy of financial resources that should be in the order of the annual budgets of some of the commercial complexes.

Networks, whilst providing a framework for the testing and application of experience gained in a particular project in other countries, can also facilitate more efficient use of scarce manpower and financial resources in participating countries through a division of responsibilities among projects. The similarity of geographical or ecological conditions between regions and a realistic compatibility of approach and flexible methodology for co-ordination between projects allows for the development of programmes of regional and international scientific co-operation (ref.27). Desirable features include problem-oriented research based on networks of interacting field projects that are interdisciplinary and complementary in nature and focus. Such a broad strategy necessitates a psychological attitudinal change in the outlook of the project planners, resource managers and research workers. An integral component is the training of endogenous manpower which should be closely linked with research and development activities in the developing regions themselves. The features of a network are at the basis of its success and these are that:

- a. The network aims to link up with concrete field projects, rather than solely with the reputations of institutes or structures
- b. Locally identified projects should have high operational character that will yield results of scientific importance and practical application
- c. The network is an open structure, continuously growing in scope and membership
- d. The planning, financing and implementation of the network projects are solely the responsibility of the local national points of contact.

The main role of UNESCO and other collaborating organizations is to provide pump-priming, supplementary support and to assist in developing links.

In addition there are three major types of interaction.

The <u>first</u> of these involves links between the projects and countries of a particular region. Modalities of regional co-operation include the exchange of people between field projects and regional training courses based on the sites of a particular project. Such interaction and collaboration is found with the MIRCENS at Beltsville, Hawaii (USA), Nairobi (Kenya) and Porto Alegre (Brazil), which are engaged in biological nitrogen-fixation programmes. Similar collaboration can be found between the Microbiological Resources Center (MIRCEN) at Bangkok (Thailand) and the World Data Centre on Microorganisms at Brisbane (Australia) in the field of culture collections.

The <u>second</u> type of interaction is the promotion of transversal (South/South) co-operation wherein measures for linking projects again include the exchange of researchers between projects e.g.

as in the exchange of research workers from laboratories in the Philippines, Thailand and Indonesia in the area of culture collection technology, or again as in the case of projects conducted by the Nairobi and Porto Alegre MIRCENS. Other mechanisms encouraging channels for communication amongst the personnel of the different networks in applied microbiology and biotechnology are through the regional newsletters and <u>MIRCEN</u> News, as well as, through the series of the International Conferences on the <u>Global Impacts of</u> <u>Applied Microbiology</u> that have been held in Stockholm (1963), Addis Abeba (1976), Bombay (1969), Sao Paulo (1973), Bangkok (1977) and Lagos (1980).

The <u>third</u> type of interaction is linkage of the MIRCEN projects with those at specialized institutions located elsewhere and with the MIRCENS themselves. Thus, there are inputs from the international agricultural research institutes, acknowledge biotechnological laboratories in Europe and North America and from professional societies. Furthermore, the MIRCENS at Guatemala, Nairobi, Porto Alegre and Bangkok are engaged in a common collaborative project that deals with fuel and biofertilizer production. Such arrangements allow for the evolution of future collaborative projects, the availability of fellowships and the joint organization of seminars and on-site training courses.

The basic tenet for the combination of training with research and demonstration for management is an understanding of the important differences in the academic environment, the prevailing socioeconomic conditions and of the availability of culture collections in different regions. This diversity emphasizes that developing countries are alert to the need of training local manpower in their own countries, and to the need of integrating such training with that of national research and development programmes. Such training also promotes a more favourable climate for scientific endeavour in many other developing countries. At present, the scientific career structure in a number of countries (with the sole merit accorded to those who publish widely in international scientific journals) does not favour the involvement of scientists in action programmes designed to solve national rural problems.

#### SOCIO-CULTURAL ASPECTS

The traits of society and their interaction with inescapable variables such as the environment, social advancement, economic progress and the like indicate that certain facets of society and culture play an important role in national development (ref.28). Socio-cultural development, in this context, can be to some extent influenced by microbial biotechnology e.g. in influencing population growth, world development, market pressures, petroleum costs and social changes. Stabilization of population growth could benefit from microbial intervention in the biosynthesis of a cheaper control product. Likewise, microbiology could provide some answers to the foreseen crisis in the area of the minerals and their availability. Similarly, market pressures in the Single Cell Protein (SCP)/Soyabean availability could change. Again, in developing alternative sources of energy, reliance and dependence on petroleum stocks could be lessened and, in the area of social changes, women and children could be spared routine demeaning tasks that prevail in some countries. For example, in the technically-advanced countries, a major social factor is the increasing emancipation of women who spend more time earning income and less time on domestic chores.

In most developing countries, the socio-cultural framework is governed by the prevailing systems of beliefs and practices that delineate right and wrong, social norms and abuses, and the interaction of religious practices with technological advancement. Again, in these countries traditional societal structures are weakened by a lack of sustained financial and skilled human resources. Moreover, age and gender in traditional societies determine the division of labour, the nature of the decisionmaking process, and the employment of skilled and unskilled labour.

Too often, only privileged segments of society benefit either socially, culturally or economically. For the poorer sections of society, the benefits that can accrue are too far away in the future. In developing countries, microbial technological applications should possess common characteristics viz: Be labourintensive, small-scale, low-cost and simple. Furthermore, the technologies available for implementation should:

- a. Benefit as wide a population as possible
- b. Be adapted to national, regional and international considerations
- c. Be attuned to environmental considerations
- d. Interweave into all aspects of daily life
- e. Be socially acceptable.

Such microbial technologies should reflect the mobilization of governmental and scientific interest and the establishment of self-reliant programmes involving:

- An improvement of traditional indigenous technology e.g. fermented foods
- b. An acceptance of a scientific once out-dated technology e.g. biogas production
- c. A revival of an old technology e.g. the acetone-butanol fermentation
- d. An adaptation to a modern technology e.g. Single cell protein feed production
- c. The promotion of regional exchange and co-operation i.e. MIRCEN network collaboration.

Technological simplicity facilitates acquisition, acceptance, diffusion and maintenance of the technology amongst the populace. Risks of dependence in operation and repair and on capital investment are reduced to the bare minimum without necessitating any inroads into meagre resources that have been earned by hard daily labour and innovation. Such simplicity, again, does not involve additional social problems nor cultural disruptions. The use of familiar materials ensures their ready accessibility and acceptability, lessens the need for "specialized training", external supervision and import of materials with its accompanying bureaucratic controls and constraints. Technological simplicity also dissolves the barriers of work organization between the rural and urban populations. In the rural areas, labour is scattered and available on the basis of kinship ties. In the urban areas, the urban poor are often engaged in specific work that is characterized more by competition rather than by co-operation. On account of the technological simplicity, and its integration into accepted

rural practices, subtle social changes are at work. Women, in most developing countries, who are apt to be illiterate, are not masters of their own time, are denied mobility and financial resources, may now have a new world opening up to them. Opportunities arise for adult education, involvement in social care programmes and voluntary health organizations. For example, in Guatemala, the acceptance of the Lorena stove by women has altered the pattern of food preparation (ref.29). Indeed, there is much truth in the statement that women hold a key position in the assimilation of new technologies as they are the transmitters of cultural values and mores to children as is evidenced from the fact that a great majority of all public advertisements geared to daily life utility products is aimed at women (ref.30).

Biogas technology is a microbial technology which addresses itself to rural socio-cultural technical and economic development. It addresses multiple national and communal needs, is fired by enthusiasm of the individuals, non-governmental organizations, local communities and above all, reflects a solid committment from government officials. Often resemblant of an Herculean effort by scientists and decision-makers to clean the Augean stables of rural poverty, integrated biogas farming is being resorted to by a number of developing countries. Reinforced by accessibility to loan-capital, technical assistance, and opportunities for rural employment and market development, integrated biogas farming involves an element of decentralization and a committed embarkation on the path of self-reliance.

A key element in the promotion of such technological developments is the cornerstone of self-reliance. Another example of a decentralized diffusion system is the famous Tachai brigade in Senshi Province. Approximately 90 households manage 144 acres of hilly land. "By constructing stone terraces, underground conduits to carry off occasional flood waters, an irrigation system, and by adopting suitable fertilizers and other agricultural innovations, the farmers of the Tachai brigade were able to raise their grain yields from 1,050 kilograms per hectare in 1949 to 5,295 in 1965 - 1966, and 8,220 in 1977. This achievement is remarkable when compared with China's national average of 3,300 kilograms per hectare.

But the main lesson of the Tachai brigade is again self-reliance. After a disastrous rain in the early 1960's washed out all of the stone terraces that had been laboriously constructed by the Tachai farmers, this brigade adopted the slogan of "three don't wants": don't want state funds for recovery, don't want state grain, don't want relief materials" (ref.31).

An important effect of such activities is a "feeling among the participants that they can control their own futures, rather than being controlled by fate, nature or supernatural forces. This feeling is reinforced by "on-the-spot-training" which is often held in conjuction with a conference or a demonstration. On-thespot training and conferences "allow participants to actually see the innovation in use, to ask how effective it has been, how to implement it, and to consider how the innovation might be utilized back home". This mechanism and technique are at the basis of the time-tested UNESCO/International Cell Research Organization courses in microbiology that have been developed through the years under the guidance of the UNESCO/ICRO Panel on Microbiology and the leadership of its Chairmen: Professors C.G.Heden (1963 - 1969), J.R.Porter (1969 - 1978), J.W.M.la Rivière (1978 - 1982) and D.G.Howell (1982 - ). The benefits of these activities have been documented and details are available in consecutive issues of MIRCEN NEWS.

## CONCLUSIONS

In conclusion, microbial technology is a field that relies on the information content of the microbe rather than on the brutal and costly forces of high temperature and pressure that are characteristic of several chemosynthetic processes. It can be applied at the full industrial scale as readily as the village or farm-scale level with promise for the developed and especially the developing countries. In this context microbial technology should be deployed in the national development policies and plans of developing countries with defined guidelines for the smallest production unit, which is in consonance with the socio-cultural environment.

#### REFERENCES

- European Federation of Biotechnology. Report of Working Group on Education--- "Manpower and Training implications of the Expansion of Biotechnology-Based Industries", Brussels, 1982.
- E.J. DaSilva, in E. Campos-Lopez (Ed.), Renewable Resources A Systematic Approach, Academic Press, London, pp. 329-368.
- D. Perlman, Chem. Technol., 1977, pp. 434-H.B. Woodruff, Science, 208 (1980) 1225-1229. 3
- S. Stewman, D. Lincoln, E. Cowden, C.H. Gunn, S. Wilcox and L. Ritter, Futures, 13 (198) 128-140. E.J. DaSilva, Acta Biotechnologia, 1 (1981) 207-246. 5
- R. Lattes, Paribas Monthly Economic Bulletin, 7 (1982) 210-216.
- 8 D. Perlman, Chemical Technol., 4 (1974) 97-108.
- G. Gregory, New Scientist, 29 July (1982) 308-310. 9
- 10 P.R. Shaw, World Development, 9 (1981) 637-655.
- 11 J. McHale and M.C. McHale, Technol. Forecasting and Social Change, 13 (1979) 97-105.
- 12 W.S. Saint, World Development, 10 (1982) 223-238.
- 13 L. Nian-guo, Asset, 4 (1982) 22-24.
- 14 V. Smil, Soft Energy Notes, 5 (1982) 88-90. 15 C.W. Lewis, M. Slesser and I. Hounam, Workshop on "Basic Needs in a Fragile Ecosystem", Praia, Cape Verde, 1981, pp.
- 16 OECD, Sector Report, The Fertilizers and Pesticides Industry, 1980, 78 pp.
- 17 T.N. Barr, Science, 214 (1981) 1087-1095.
- 18 D. Plucknett and N.J.H. Smith, Science, 217 (1982) 215-220. 19 G.S. Venkataraman, Current Science, 50 (1977) 253-256.
- 20 G.S. Venkataraman, Algal Biofertilizer for Rice, IRRI, New Delhi, 1977, 7 pp.
- 21 L.A. Falcon , in M.D. Summers and C.Y. Kawanishi (Eds.), Viral Pesticides - Present Knowledge and Potential Effects on Public and Environmental Health, C.Y. EPA, North Carolina, USA, 1978, pp.
- 22 K. Bosecker and M.Kursten. Process Biotechmistry, 13 (1978) 2-
- 23 C.A. Brierley, Scientific American, August (1982) 42-51.
- 24 V.G. Desa, Impact. Sci. Society, 28 (1978) 105-116.
- 25 Anon., J. Biol. Education, 16, pp 10-12.
- 26 E.J. DaSilva in S.O. Emejuaiwe, O. Ogunbi and S.O. Sanni (Eds.), Proceedings Sixth International Conference on the Global Impacts of Applied Microbiology, Lagos, Nigeria, Academic Press, London, 1981, pp. 547-554.
- 27 F. Dicastri and M. Hadley, Fifth International Symposium of tropical Ecology, Kuala Lumpur, Malaysia, 16 - 27 april, 1979, published?
- 28 E.J. DaSilva in Proceedings of the EEC/UNESCO/CNRS Colloquium "L'Impact des Biotechnologies sur le Tiers Monde", Paris, 1982, pp. 195-209.
- 29 D.V. Shaller in Lorena Owner-Built Stoves, 2nd ed., Volunteers in Asia, Stanford, California, USA, 1979, pp.
- 30 O.C. Menot, Personal Communication
- 31 BOSTIC, Diffusion of Biomass Energy Technologies in Developing countries, National Academy Press, Washington, D.C. 1982, pp.

# THE BIOTECHNOLOGY SITUATION IN INDIA - BIOTECHNOLOGY FOR VILLAGES

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#### ABSTRACT

This paper is, in the main, in two parts. The first part reviews the biotechnology situation in India as the region in South Asia where an important part of the activity in this field seems to be concentrated. The second part of the paper deals with our own work on biotechnology in rural areas. Several colour slides shown during the Symposium are not included in this article.

#### BIOTECHNOLOGY ON THE NATIONAL LEVEL IN INDIA

# Official initiatives and teaching

The importance of biotechnology has been recognised, on the national scene, with the formation of a National Biotechnology Board and so far the Board has catalysed the formation of two major institutions, one for Cellular and Molecular Biology and one for Immunology. The Board has also taken steps to bring back Indian scientists living abroad. India is organizing the International Biotechnology Symposium in February 1984. The special symposium on Yeasts has been held separately in early 1983 in Bombay.

Bilateral and multilateral agencies play an active role in Indian biotechnology. Perhaps the most tangible applications of biotechnology in the field have been provided by the All-India Coordinated Programmes on Blue-green algal fertilizer (ref.1) and Rhizobium applications. The programmes have a national character and are being implemented over millions of hectares of land. All the programmes have or have had some component of international assistance by way of funds.

In the public and official mind, biotechnology is firmly filed as the discipline of practising microbiologists, and traditional bioengineers have so far been left out of the mainstream of policy making. Leading schools of biotechnology exist in India, and the discipline owes a great debt to T.K. Ghose who has pioneered teaching and training in India; in addition to the Indian Institute of Technology, Delhi where he teaches, there are several schools on the subject. India has considerable manpower in the computer sciences, systems technology and biological sciences. Thus there is no shortage of associated expertise to implement large-scale biotechnology programmes. However, man-power quality is a very difficult virtue to keep up to the required competence over some years.

# Industrial applications and research

In the area of biotechnology, the multinational corporations are less dominant than formerly; however, recently there has been a number of incentives for foreign companies to enter into technological agreements. As the former Chief Technical Executive of South India's only Yeast Factory, the author feels that technical collaboration agreements are extremely difficult to execute in spirit and in word because of the many obstacles to development, both bureaucratic and otherwise. Too many state and central governmental procedures have conflicting requirements, not to speak of purely local interests.

The practice of biotechnology on the industrial scale is quite extensive in India. About 400 000  $m^3$  of alcohol is produced per year in 127 distilleries (installed capacity is 750 000  $m^3$ /year). Only cane molasses are used as a substrate. Ten new plants, to use cassava as a substrate and to produce 2000  $m^3$ /year each, have been licensed. It is recognised in India that alcohol will continue to be more useful as raw material for chemicals than as fuel. Already a number of alcohol-based industries exist and this situation is likely to expand. Cane molasses are also a substrate for the citric acid industry. There is no substantial activity in the food or fodder yeast industry with the exception of the factory started by the author. There are many factories making baker's yeast.

The only other large-scale industrial practice of note is the antibiotics industry. With a yearly capacity of 600 million mega units of penicillin, 400 tons of streptomycin and 200 tons of oxytetracyline, India is very well equipped in this area. The Hindustan Antibiotics Limited (Government) is the largest undertaking. Recently there has been a relaxation of rules to permit private manufacture of nine bulk drugs, which is bound to stimulate a great deal of activity.

The Government of India gives considerable tax incentives for industries to do research in renewable resources for energy and in other thrust areas in biotechnology. Thus there is considerable activity in this area in the private sector. Perhaps the most significant recent finding in India (ref.2), done privately, is the development of a culture medium for the leprosy bacillus, which opens the way to the development of a vaccine for this widespread disease.

There has been a great deal of research activity in the areas of nitrogen fixation, bioconversion processes for energy and chemicals, and drug production. Techniques ranging from genetic engineering to tissue culture are of great interest, and many projects exist in these areas.

## BIOTECHNOLOGY FOR RURAL AREAS

# Utilization of water hyacinth

The basic policy of the Shri A M M Murugappa Chettiar Research Centre has been to integrate various biotechnologies at the rural level. The many uses of the water hyacinth plant are shown in Figure 1, as one example of the variety of ways a local residue can be used. Which of these pathways is the best and how can this best be determined?

As shown in Table 1, the biological variability of the harvested leaves is an important factor: mature and tender leaves differ in their protein content as well as in the amount of biogas that can be produced from them per unit dry weight. The photograph, Figure 2 shows water hyacinth leaves being harvested from a catamaran.

A major recent observation (ref.4) is the discovery of an endogenous photosynthetic bacterium (PSB) in the shoot apex region of the stem of the water hyacinth. It is known that such bacteria are





Fig. 1. Possible utilization of water hyacinth.

useful for sewage treatment, nitrogen fixation, hydrogen production and so on. The Centre is exploring the possibility of adapting the PSB to another wet crop, namely paddy. Preliminary experiments show promise. Crushed water hyacinth juice, in fact, seems to work through its endogenous bacteria.

#### TABLE 1

Age of water hyacinth leaves in relation to protein and gas production.

Mature and tender leaves distinguished only by visual inspection of leaves from the same pond.

Results of last three parameters obtained from experiments where protein was not separated after 16 hours.

The entire biomass was allowed to ferment for 35 days.

and the second second	Mature	Tender	1.11
moisture %	92	94	- 22
dry weight % in wet			
leaves	8.25	5.95	
crude protein % of			
dry weight	22.4	15.75	
protein concentrate			
weight, g	0.995	0.65	
protein, %	52.5	35	
yield, g/kg dry leaves	142	130	
gas production, litres			
per kg wet leaves	13.79	4.42	
protein concentrate,			
g per kg dry leaves	98.12	90.0	
crude soluble protein		5e	
in supernatant, g	2.44	0.945	

#### Mass cultivation of algae

The mass culture of algal species has been the subject of intense interest in India (ref.5; 6). We have, as Table 2 shows, worked out the cost of a backyard activation system for <u>Spirulina</u> <u>fusiformis</u> for protein enrichment. The idea is that in 10 m<sup>2</sup> of home ponds each person should become (almost) completely selfsufficient in protein preparation, with some expertise provided by a central laboratory. The situation of self-sufficiency is similar to that with regard to artificial insemination centres which are now fairly widespread. The microbial expertise should be provided by a central laboratory.


Fig. 2: Water hyacinth leaves being harvested from a catamaran.

#### TABLE 2

Backyard algal cultivation for protein

- production of algae 100 gm per day for a family of 5
- this will meet 50% of the protein requirement of a person area requirement 10 m<sup>2</sup> (say a pond of 5 m x 2 m)
- culture volume 1000 litres
- culture span 90 days
- medium: ½ Zarrouk medium containing bicarbonate, phosphate, nitrate, sea salt
- assuming initial chemical cost and other requirements to be subsidised, costing for only replenishment chemicals it works out to 14-20 Indian rupees per kg of dry algae
- or 0.29 Indian rupees per person per day which is less than the amount he is likely to spend on vegetables every day

Another concept that is partially implemented is the Algae-Milk Farm (ref.7). Here the idea is that a plot of land optimises its resources, including water, to yield algae and milk. Biogas from dairy cattle is the main energy source. As shown in Figures 3 and 4, the farm relies on integration of all the biotechnologies to produce milk and algae. Figure 4 is the carbon and nitrogen balance for a 10 kg/day algae production.

#### Biogas plants

Biotechnology at the village level cannot ignore biogas plants. MCRC, for its part, has been engaged in developing a low-cost design by using a PVC or low-density polyethylene sheet. The advantages are its simplicity for fabrication and handling. The cost economics are shown in Table 3.

#### TABLE 3

Comparison of different digester designs capacity 3 m<sup>3</sup>

	Indian drum type	chinese fixed dome	mcrc balloon type
diameter (m)	1.35	2.8	1.56
depth (m)	3.0	2.3	1.79
retention time (days)	55	60	30
gas holder	mild steel	masonry	LDPE balloon
bricks	2900	2200	2000
cement (kg)	750	700	600
cost <sup>X</sup> (rs)	3100	1800	1300

\* = based on 1980 rates





#### Assessment of value systems

Thus far we have been discussing the applications of biotechnology in rural areas. However, the application of any kind of technology is not value-free and it is high time that we had a serious discussion about the value systems associated with technology. We place the problem in the perspective of a recent statement (ref.8), connected with the negative effects of technology transfer, in particular agricultural technology:

- "- Food aid policies that can make some developing countries more dependent on North America and relieve them of the necessity for bolstering their own agriculture.
- Introduction of food habits that cannot be sustained by local food production, such as wheat bread and wheat products, into tropical countries that cannot produce wheat and at the expense of local foods that they can produce.
- The overly agressive promotion of infant formulas in some developing countries that has contributed to the decline in breast-feeding, although this is not the primary cause of such a trend as some assert.
- Large-scale production of cash crops in ways that do not benefit the small farmers and mean only limited, low-paid employment for plantation workers.
- Support of mechanization that displaces farm laborers and also tenant farmers, as well as the buying up of small plots. The net effect is to increase the number of landless laborers and unemployed, and to stimulate further migration to cities unprepared to receive them.
- That a significant proportion of the foreign technology introduced into developing countries at a foreign exchange cost is not really needed, is already available in local institutions, or is actually undesirable".

We in India share this kind of problem with many of the developing countries in most areas of technology. Why is this so? And how does it affect our rural areas? This is because commercial markers are the sole means of judging the success or failure of a technology, and as long as this persists in a basically non-market economy, the value system will be skewed towards the moneyed interests. The intangible benefits of a technology are never weighted properly, if for no other reason, because there is just no way of assessing the difference between something that is worth cash and something that is for the public "good".

Fig. 4: Carbon-nitrogen balance for 10 kg/day of algae. \* = Metabolic use



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#### REFERENCES

- 1 All India Coordinated Project on Algae, AICPA Annual Report, New Delhi, India, 1979-1980.
- 2 N.Veeraraghavan, Research Publication, V.H.S. Medical Centre, Madras, India, 1982.
- 3 C.V. Seshadri, Bhama S. Rengan and B.V. Umesh, Proc. National Solar Energy Convention, Solar Energy Society of India, Annamalainagar, India, 1980, pp. 70-71.
- 4 C.V. Seshadri, N. Jeeji Bai, R. Manoharan and Bhama S. Rengan, Microbios Letters, 1982, pp. 25-33.
  5 C.V. Seshadri and Sebastian Thomas, Proc. National Workshop on
- 5 C.V. Seshadri and Sebastian Thomas, Proc. National Workshop on Algal Systems, Indian Society of Biotechnology, III, New Delhi, India, 1980, pp. 175-179.
- 6 C.V. Seshadri, Sebastian Thomas, R. Manoharan, N. Jeeji Bai and G. Raja, MEPS, MCRC, Tharamani, Madras, 113, India, volume 5, 1980.
- 7 Staff, Murugappa Chettiar Research Centre, Proc.National Solar Energy Convention, Solar Energy Society of India, Annamalainager, 1980, pp. 68-69.
- 8 Nevin S. Scrimshaw, World Development vs Hunger, Cambridge Forum, Cambridge, Mass., USA, 1982.

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THE STATE OF BIOTECHNOLOGY IN AFRICA, WITH SPECIAL EMPHASIS ON BIOLOGICAL NITROGEN FIXATION

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#### ABSTRACT

Traditionally, Africans have used microorganisms deliberately to produce beverages, and incidentally enjoyed the benefits of microbial activities in such areas as the food industry, in hide tanning and soil fertility maintenance. There are often marked differences in technologies employed in any one process and the lack of communication and mass adoption has hampered progress. But there should now be a concerted effort by organizations such as the African Regional Centre for Technology to orchestrate the change from the many small-scale, uncontrolled technologies to more appropriate, more scientifically-based technologies.

In the first part of my paper, the kinds and status of biotechnologies in three areas which have received or are now receiving a lot of emphasis have been selected for more detailed discussion. These are waste recycling, biogas production and the role of microbiolo-gy in traditional fermented foods and beverages. In waste recycling, the quantities of industrial, agricultural and municipal wastes produced are presented, and current and future uses are discussed, with an emphasis on the optimization of the microbial reactions involved. Alcohol production is receiving major emphasis. In biogas production, its present state in 10 countries will be discussed, with Tanzania receiving more emphasis because well thought-off schemes including estimated costs of setting up plants for rural biogas production have been drawn up. In the area of fermented foods and beverages data are presented from several countries, which show that Saccharomyces cerevisiae and lactobacilli are the two most prevalent, if not most important, industrial microorganisms on the continent. This is true for the rural areas as well as for the urban areas where many of the new biotechnology plants are sited.

In the second part of my paper, I review the state of food crop production in Africa, with special reference to biological nitrogen fixation (BNF) technology. Diminishing food crop production is the major problem facing Africa today, and the situation is forecast to worsen in the next two decades. BNF, mycorrhizal fungi and microbial control of insect pests and plant pathogens are major areas requiring urgent attention and financial support from African governments. Modest gains have been made in the area of BNF, principally, because African microbiologists have succeeded in adopting and applying technologies that have been developed in industrialized countries. BNF technology is now being applied towards

production of food crops in countries such as Egypt, Zambia, Zimbabwe, Kenya and the Ivory Coast. The relative inexpense and the role of regional and international bodies in BNF technology will be examined; the expected impacts on African agriculture will be discussed. Priority areas in BNF, i.e. the Rhizobium/Legume symbiosis, Azolla/Anabaena symbiosis and blue-green algae, will be discussed in relation to arable crop farming, regeneration of forests and environmental protection.

In the third part of my paper, I present what the future portends - the biotechnologies requiring attention and the organizational structures required to promote biotechnology in Africa.

#### INTRODUCTION

Microorganisms have been used for the benefit of the African for many centuries. Examples include fermentations in food and beverages, leather tanning, and maintenance of soil fertility. They have also been used as food, e.g. Spirulina by inhabitants of the Lake Chad region. Unfortunately, in several cases, the microorganisms responsible have not been isolated for mass culture and so many of the processes continue to be operated on a small scale. As one travels from country to country, and at times from locality to locality within the same country, one encounters very different techniques for the same process. Whereas this sort of variation most likely arose from differences in ecological conditions and availability of raw materials, there is now clearly a need to have more uniform conditions employing well described microbial species, to enable the technological take-off necessary for these times. The scientific awareness and know-how are often lacking and these need to be corrected as a prerequisite to placing Africa on the path to a better and more sound exploitation of microorganisms and their capabilities for technological development.

In my presentation, I will first review some published information in four areas of biotechnology:

1. Waste recycling,

- 2. Biogas,
- 3. Foods and beverages, and

4. Food crop production.

Subsequently, I will dwell at some length on biological nitrogen fixation, a process that plays a key role in crop production and soil fertility maintenance. I will discuss its status in Africa today, the countries and institutions where major research and 72

application are under way, and the agencies that support work on it.

Finally, I will discuss the objective of the Organization of African Unity (OAU) and the African Regional Centre for Technology for promoting Africa's scientific and technological development.

#### WASTE RECYCLING

The three levels of development - village, town and city - have different waste production and disposal methods and attitudes (ref. 1). Thus, under the traditional, rural form of life, there are usually few wastes to worry about. With urbanization, industrialization and increased farm outputs, the kinds and quantities of wastes increase. The traditional way of viewing wastes as nuisances have now given way to the concept of recycling; wastes are now a resource. The by-products shown in Table 1 have much promise as substrates for microbial processes in developing countries.

#### TABLE 1

## By-product substrates

Agricultural	Other
Molasses	Animal manures
Bran	Sewage
Bagasse	Municipal garbage
Oilseed cakes	Industrial effluents from
Bark	- paper mills
Straw	- canneries
Coffee hulls	- fisheries
Cocao hulls	- slaughter houses
Fruit peels and leave	es - milk processing plants
Cotton wastes	
Tea wastes	
Sawdust	

Many of these occur in abundant quantities in Africa. The kinds of quantities of wastes produced in Egypt (Table 2) and Nigeria (Table 3) illustrate the point. Municipal wastes in Nigeria are for the capital city, Lagos, only, where over \$30 million are spent annually to burn the "wastes" (ref.2). Also there are now well over 47 breweries in Nigeria; the quantities of wastes provided are for one brewery (ref.3). TABLE 2

Wastes in Egypt (ref.7)

Source	Waste	Tons/yr.
Sugarcane	Fodder veast	5,000
Farm	Maize cobs	600,000
	Bagasse pith	60,000
	Rice hulls	250,000

TABLE 3

Wastes in Nigeria (ref.4,ref.5).

Source	Waste	Tons/yr.	
Breweries	Spent grains	11,500	
	Yeast slurry	300	
Fruit industry	Peels, etc.	25,000	
Municipal	Garbage	480,000	
Sewage	Night soil	20 M gal	
Sugar industry	Fodder yeast	7,000	

Wastes are recycled into many useful end-products in Africa. An FAO survey (Table 4) showed that wastes are used in the production of animal feeds and several industrial end products.

#### TABLE 4

Agricultural by-products and wastes in Africa (FAO, 1973, ref.6)

Cou	ntry	Raw materials	End-products
1.	Algeria	milling industry rape seed cake grape waste	Animal feed, grape seed oil
2.	Botswana	Slaughterhouse waste	
3.	Burundi	Cotton seed cake Palm kernel cake Molasses, rice bran	Animal feed
4.	Cameroon	Seed cakes Cocao pods, brewery wastes, coffee pulp bananas	Animal feed
5.	Chad	Animal by-products oil cakes	Blood meal, Compost, *N fertilizer Animal feed

# Table 4 continued

Cou	ntry	Raw materials	End-products
6.	Congo	Sesame + cake, groundnut cake wheat bran	Animal feed
7.	Egypt	Fruit & Vegetable wastes Sawdust, cigarette fil- ter wastes Dairy products Molasses, bagasse Onion wastes, mango seed	Animal feed *Alcohols *Acetic acid *Yeast *Acetone Acetates Oils Starch
8.	Ghana	Wheat bran bagasse, molasses Cocoa pods brewery wastes, banana Palm, Cassava Animal by-products	Animal feed Pectin
9.	Ivory Coast	Coffee parchments Cocoa pods Fruit & vegetables Root & tuber crops	Starch Animal feed
10.	Kenya	Coffee skins, pulp Sisal waste Groundnut haulms	*Methane *organic fert. Animal feed Caffein Oil
11.	Madagascar	Manure Apple & pineapple wastes bagasse	Human food, Animal feed *Compost
12.	Malawi	Wood product residues Fisheries residues Cotton, legumes	Animal feed
13.	Mauritius	Sugar cane - by products bagasse	Protein Animal feed *Microbial protein *Methane gas
14.	Morocco	Beet sugar by-products Pulp Molasses Brewery wastes Oil press cakes Casein, Whey Municipal waste	Fertilizer *Alcohols *Yeast Sugars Oils Feeds Pharmaceuticals
15.	Niger	Sorghum Millets	-

#### Table 4 continued

Cour	ntry	Raw materials	End-products
16.	Nigeria	Cassava waste Cocoa waste brewery waste Groundnut cake Oil palm wastes Fruit wastes	*Single cell protein *Compost Animal feed *Fuel
17.	Senegal	Millet, sorghum & groundnut	Animal feed *Compost
18.	Sierra Leone	Sesame seed cake, wheat mill feed and rice bran	Animal feed
19.	Somalia	Animal by-products Bagasse & molasses Fruit	Animal feed *Alcohol germs, bran
20.	South Africa	Sugar industry waste Cheese whey Fruit wastes Municipal wastes Industrial wastes	*Yeast protein *Tartrate *Alcohols *Compost Oils
21.	Sudan	Sugar industry wastes Slaughter-house by- products tannery wastes brewery wastes Straws	Animal feed
22.	Tunisia	Wheat by-products Fish by-products Sugar industry wastes Fruit wastes	Animal feed *Yeast *Alcohols *Organic acids *Compost
23.	Uganda	Brewery waste Sorghum and millet residue	Dairy products Poultry feed
24.	Zaire	Cassava, brewery wastes, dairy wastes	
25.	Zambia	Molasses	Animal feed

\* Microbial process involved.

In Egypt, microorganisms metabolize various industrial wastes to yield single cell protein (Table 5).

#### TABLE 5

Production of single cell protein in Egypt from various industrial wastes (ref.7).

Industry	Microorganisms
Sugar	Candida spp.
Cellulose	Candida spp.
Food	<u>Toluropsis</u> <u>candida</u> <u>Saccharomyces</u> <u>fragilis</u>
Petroleum	Candida lipolytica C. tropicalis

By far the most active area of biotechnology in Africa today (aside from brewing of beers) is the production of ethanol from sugar cane wastes. Several African countries spend a major portion of their foreign exchange to import oil. In the case of some, like Tanzania, this amounts to over 60%! (ref.8). There is therefore good reason to decrease this dependence on petroleum. Ethanol factories have been in operation in Egypt, Morocco, Tunisia, South Africa and Zimbabwe already. Other countries are planning to join the group. In Malawi, the Ethanol Company Limited was to save the country U.S. \$2 million annually when, late last year, it was to commence ethanol production. The plant was to cost \$9.31 million (ref.9). In Mauritius, an Austrian loan of \$11.6 million would enable the construction of a plant with a capacity of 60,000 liters/ day (ref.10).

Tanzania's first power plant is slated to go into production in 1985 (ref.8); it will have an annual capacity of 10 million liters. The plant will be located in Moshi, 14 km away from the Tanganyika Planting Company, where 25,000 tonnes of molasses are produced annually. The plant will cost \$12.51 million, with \$8.67 million being a loan from France. The ethanol, when mixed with gasoline, will reduce Tanzania's oil import bill by 10%. National Chemical Industries and the Sugar Development Corporation will jointly run the plant.

Zambia aims to cut down its oil import bill by 8% when it produces 12 million liters of ethanol annually, at a daily rate of

65,000 liters. The plant will cost \$18.21 million, to be financed by 8 local and international investors, including the Barclays Bank, Anglo-American Corporation, Tate and Lyle, and the European Investment Bank. The plant will be located at Nakambala Sugar Estates and will be jointly run by Jager and Associates of Zimbabwe and Ethanol Company of Zambia (ref.11).

Kenya is building an alcohol plant in Kisumu, to go into production in 1982. It will cost over \$100 million, most of this being foreign loans; the government will however own 51% of the company. The plant is estimated to save Kenya \$7 million in foreign exchange annually. Molasses will serve as substrate for producing 20 million liters of alcohol yearly, to be used as a high octane substitute for imported petroleum. By-products from the process will include (ref.12):

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citric acid	-	3,000	tonnes/year
yeast	-	1,800	
vinegar	-	2,160	,,
oxygen	-	6,800	
sulfuric acid	-	7,500	
ammonium fertilizer	-	2,000	
gypsum	-	6,000	
carbon dioxide	-	9,000	

Basically, there are two substrates in tropical countries that can be realistically considered for biomass conversion on a large scale. These are sugarcane and cassava. From the foregoing discussion, it is evident that African countries depend on by-products of sugar cane processing as the substrate. But, whereas sugarcane can yield between 60-75 liters of ethanol per tonne, cassave tubers can produce about two-and-one-half times this volume (ref.13). Cassava yields of more than 40 tonnes/ha are now feasible in Africa, so this area needs greater attention. Competition of fuel needs and food needs for cassava must be borne in mind. Brazil has experience in gasohol production from cassava and can provide assistance in that area. Berkovitch (ref.14) has indicated that four basic stages are involved:

- 1. Extraction of starch from cassava;
- 2. Liquefy, saccharify and ferment starch slurry;
- Primary distillation producing 50% alcohol from a feed of 9% alcohol, and

4. Azeotropic distillation, including rectification, yielding alcohol of fuel grade from feed of 50% alcohol.

The first two stages can be carried out at the farm or village level, but the last two must involve modern industrial distillation for optimal yields.

#### BIOGAS

New technologies in developing countries tend to be sited near urban centers. Fortunately, biogas technology is one that is excepted from this because it is well suited to rural areas.

Biogas is produced by the anaerobic decomposition of excrements and plant materials by microorganisms in a three-stage process (ref.14), viz.

- Stage 1. Production of <u>soluble compounds</u> from fats, cellulose and proteins by microbes active on these substrates.
- Stage 2. Production of <u>organic acids</u> (mainly acetic) from the soluble compounds by acid bacteria.
- Stage 3. Production of methane and  $CO_2$  from the acids by methanogenic bacteria. The ratio of methane to  $CO_2$  may be as high as 60:35.

Raw materials for biogas production include cattle dung, piggery and poultry droppings, animal manure, human excrement and several agricultural wastes. According to Reynolds (ref.15) the waste materials required to produce at least 50% methane gas (600 BTU/cu. ft.), should have a C/N ration of 30:1. This figure is rarely obtained. Some possible substrates and their C/N ratios (ref.14,16) are:

cow manure	:	18 - 25
horse manure	:	20 - 25
pig manure	:	15
poultry manure	:	7 - 15
human excrement	:	25 +
grass cuttings	:	12
green vegetable waste	:	12
straw	:	128 - 150
sawdust	:	200 - 250

A report by Odeyemi (ref.17) showed that <u>Eupatorium odoratum</u>, a highly productive, ubiquitous weed, was superior to water lettuce and cowdung, yielding 256 liters of biogas/kg. This value is higher than that obtained from other sources also (Table 6). In Tanzania, with 65 cows per 100 inhabitants, cow dung has been found to yield up to 13 cu. ft. of biogas per animal per day, which is nearly twice as much energy as would be obtained by the traditional method of burning dung cakes (Table 7). There is also the added advantage of harvesting fertilizer from the residues of the digestion.

#### TABLE 6

Gas production from various raw materials (from Lyamchai and Bahtia, 1980 (ref.18)).

Type of animal	Availability kg/day	Gas/kg (cu.ft.)	Gas/animal/day (cu.ft.)
Cattle	10.0	1.3	13
Pig (45 kg)	2.25	2.8	6.3
Poultry (2 kg)	0.18	2.2	0.4
Nightsoil (excreta)	0.40	2.5	1.0

TABLE 7

Comparative benefits in a year using 45 kg of fresh cattle dung per day (ref.19).

Method of utilization	Fuel obtained	Effective heat value	Manure obtained	
Farmyard manure	-		7 cartloads	
Cowdung cakes for fuel	3.65 t	850,000 k cal	and the second	
Gas plant digestion	620 m <sup>3</sup>	1,870,000 k cal	10 cartloads	

Biogas plants were either under construction or in use at the pilot stage in these 11 countries in 1978 (ref.20): Algeria, Cameroon, Congo, Ethiopia, Kenya, Rwanda, Senegal, Tanzania, Upper Volta, Zaire and Zambia. Illustrations and descriptions of the kinds of digestors in these countries are provided by Mayele (1978). Tanzania, through its Small Industries Development Organization (SIDO), seems to have taken a leading role in this area of biotechnology. There are over 80 plants in Tanzania (ref.18). There was a meeting there recently, attended by 35 participants from 14 countries and representatives of UNIDO, UNESCO, IFS, SIDA, and others (MIRCEN News, ref. 20). Discussants from Botswana, Ethiopia, Kenya, Rwanda, Sudan, Tanzania and Upper Volta mentioned experiences with the batch-type digestors. Botswana has studied the performance of fixed and floating dome digestors, and concluded that the fixed-dome type had several disadvantages, the main one being leakage of gas, through the concrete layer at the joints of the dome. It was concluded that the good performance of the fixeddome type depended on the availability of a good mason. This conclusion was borne out by the Sudanese experience. Hols (ref.21) gives the problems and advantages of batch and continuous digestors.

The participants to the meeting recommended that any country wanting to set up a biogas plant needed to also set up a national biogas technology center for research and development, training and to liaise with extension workers and other national centers.

Egypt is another country that is very much interested in biogas technology. There, a 5-year USAID sponsored biogas technology project has been examining the technical, social and economic feasibility of biogas technology since 1978 (ref.22). Several Indian and Chinese digestors are being evaluated in terms of cost of construction, gas composition etc. Subsequently, demonstrations will take place in two villages prior to possible implementation at the national scale.

The economics of using materials from various sources in digestor construction and gas production have been worked out, based on 7 countries of Asia and the USA (ref.23). In Africa, Tanzania has provided an estimate for a sample plant (ref.18), as follows:

Capital cost	:	US	\$110
Operating cost	:		238
Annual returns	:		332
Net returns			11.

Based on the Tanzanian experience Lyamchai and Bhatia (1980, ref.18) have enumerated six points that will determine how well biogas plants will be accepted and can be sustained:

- The cost of installation and maintenance of gas plants is a major bottleneck. The cost of steel parts, especially, could be a problem.
- 2. Lack of technical experts and servicing facilities for maintenance of plants at the rural level. Training is required.
- 3. Campaigns to popularize plants are required.
- Specific gas burners and gas stoves are required at cheaper prices and in larger numbers.
- 5. Low temperatures in some regions and at certain times of the year could be an important hurdle.
- 6. Village models now in use are economic for a family of 6-7 members, and possessing 5-6 healthy adult animals; larger models for more people are required.

#### FOOD AND BEVERAGES

Since it is generally accepted that the first deliberate industrial use of microorganisms was in the brewing industry, one must also conclude that the brewing industry in Africa has recorded the longest history of biotechnology. Many African foods are fermented to preserve them and to impart certain desirable flavors. Thus, the use of dried yeasts in beer production and the use of soured water as a starter culture in fermented millet porridge are examples showing that the African has always been aware of the role of microorganisms in these processes.

The recent reviews by Okafor (1980, ref.24), Nout (1980, ref.25) and Abd-El-Malek (1978, ref.26) have provided an excellent basis for my discussion of the uses of microorganisms in African foods and beverages. There are basically five categories of fermented foods and beverages, made from either milk or carbohydrates. The carbohydrates include the root of cassava (<u>Manihot esculenta</u> Crantz), cereal grains such as maize (<u>Zea mays</u>), sorghum (<u>Sorghum bicolor</u>) and millet (<u>Eleusine corecana</u> and <u>Pennisetum</u> sp.) as well as the palms (<u>Elaeis</u> sp., <u>Raphia</u> spp.) and the coconut (<u>Cocos nucifera</u>). In many parts of central and East Africa, bananas and plantains are carbohydrate sources for fermentations.

#### 1. Milk and milkproducts

In Egypt and several North African countries as well as among the nomadic herdsmen of West and East Africa, fermented milk cheese and other milk products are principal components of the diets. The reviews of Abd-El-Malek (ref.26) and Nout (ref.25) show that the principal microorganisms are homofermentative lactobacilli, Leuconostocs, yeasts and streptococci. About half of the milk produced in Kenya is consumed at home as fermented milk (Table 8). Fermented milk products account for more than 70% of the lactic acid fermentations in Kenya (Table 9).

#### TABLE 8

Kenya: estimated yearly milk production and processing for human consumption (1977/78) (ref.25).

¥ 11	Volume (10 <sup>6</sup> liters)	Per cent
Total yearly production	1400	100
Withheld for home consumption - consumed as fermented milk - consumed fresh	748 402	53 29
processed by dairy industry - into fluid milk products - into other dairy products	250 100	11 2

#### TABLE 9

Kenya: estimated consumption of lactic acid fermented foods (pop. approx. 15 m) (ref.25).

Product	Consump- tion(kg/ year)	Average %Acidity lactic acid	Equiv. pure lactic acid (kg)
Fermented milk products (1978)	$7.5 \times 10^8$	1.25	9.5 x 10 <sup>6</sup>
Fermented cereal products: "uji" gruel (1977) "busaa" opaque beer (1978)	6 x 10 <sup>8</sup> 3 x 10 <sup>8</sup>	0.25 0.70	1.5 x 10 <sup>6</sup> 2.1 x 10 <sup>6</sup>
Other fermented products: brewer's spent grains (1980) vegetables (1980)	3 x 10 <sup>6</sup> 5 x 10 <sup>6</sup>	1.5	4.5 x 10 <sup>4</sup> 75
Total			13 x 10 <sup>6</sup>

# TABLE 10

Fermented foods of Africa South of the Sahara (From various sources by Okafor, 1980 (ref.24).

Name of food	Where consumed	How processed	Level of Advance	Microorganisms
		Cassava-based		一部 王 王 王 王 王 王
Gari	West Africa; Zaire	Pulp fermented	1,4,6,7	Leuconostoc Streptococcus yeasts
Foo-foo	Nigeria	Whole roots fermented	0	
Chikwangue	Zaire		0	· 3 · · · · · · · · · · · · · · · · · ·
Lafun	Nigeria	Flour from chips	0	and the second sec
Kokonto	Ghana		0	
Cingwada	East, Central and South Africa		0	
		Cereal-based		
Non-Alcoholic				
Ogi	Nigeria, Benin Republic	Fermented ground cereal	1,2,4,6 7, (8?)	<u>Lactobacillus</u> yeast
Koko (aflate)	Ghana	A DECEMPTER AL MUNICIPALITY	1	Lactic acid bacteria
Mahewu (Mogow)	South Africa		1,2,4,5 6,7,8	<u>Lactobacillus</u> delbrückii
Alcoholic			1.4	
Burukutu/Pito	West Africa	Fermentation of malted sorghum	1,2	Lactic acid bacteria,yeasts
Kaffir beer	South Africa	··· 24	1,2,4,5 6,7,8	Lactic acid bacteria Saccharomyces

Table 10 continued

Name of food	Where consumed	How processed	Level of Advance	Microorganisms
		Palm-based		
Palm wines	East, West, Central and South Africa	Spontaneous fer- mentation of palm sap	1,2,7	Lactic acid bacteria, yeasts, acetic acid bacteria
		Miscellaneous		
Iru (dawadawa)	Nigeria	Fermented seeds of Parkia	0	
Ogili	Nigeria	Fermented seeds of castor oil	0	:
Ugba (Ukpaka)	Nigeria	Fermented seeds of oil-bean	0	
Fura (Ghussub)	Mali Nigeria	Millet and cheese	0	
Amasi	Easte, Central and South Africa	Fermented milk	0	

Key: 1 = Organisms isolated

- 2 = Role(s) of organism(s) determined
- 3 = Selection and genetic improvement of organisms
- 4 = Process improvement

- 5 = Improvement in raw material used
- 6 = Laboratory simulation of fermented food
   production
- 7 = Pilot plant production
- 8 = Industrial plant production

#### 2. Cassava-based fermented foods (see Table 10)

- (a) Anaerobic fermentation. Whole or cut cassava roots are allowed to rot for a while. The softened roots are peeled, macerated and sieved to yield a starchy material. This is boiled, at times wrapped in leaves. Often, boiling is done more than once, with pounding between each boiling. In Nigeria the food from this rotted root is called <u>foo-foo</u> and in Zaire, <u>chikwangue</u>. The organisms have not been identified but are probably clostridia, based on the strong odor of butyric acid. The fermentation and boiling processes destroy the cyanogenic glucosides in certain cassavas.
- (b) Flour from partially-dried chips. Chips made from peeled cassava are fermented for a short period of 24-36 hours, sundried, ground and sifted. The resulting flour is cooked into a paste in water, and is consumed along with a stew. The food is called <u>kokonte</u> in Ghana, <u>lafun</u> in Nigeria, and <u>cingwada</u> in Kenya and Malawi. The organisms involved have not been described.
- (c) Fermented, grated pulp, <u>garri</u>. Cassava is grated and fermented while the pulp is being dehydrated, usually with the aid of heavy objects placed on the cloth bag bearing the pulp. The fermented pulp is then heated in a dry pot, with or without palm-oil to produce white or yellow garri. The process also detoxifies cyanides in cassava. The organisms involved are lactic acid bacteria (<u>Leuconostoc</u> sp. and <u>Streptococcus</u> sp.) and yeasts. Garri is the only food for which Africa has developed a patented plant (UN, 1978, ref.27). The Federal Institute of Industrial Research, Oshodi, Nigeria has designed plants with capacities of 100-200 lbs/day for the village level, and of 10 tons/day. Similar plants have been produced and are in use in Ghana.
- 3. Cereal-based fermented foods (see Tables 10 and 11)
- (a) Non-alcoholic. Fermented cereal foods are made by first soaking the grains for 24 hours. They are then ground and sieved and the starchy material is fermented a further 24-72 hours. <u>Ogi</u>, consumed in Nigeria and Benin Republic is prepared in this manner. <u>Koko</u>, eaten in Ghana, is prepared in a similar manner except the ground material is not sieved. Both are important breakfast porridges and weaning foods. Both can be converted to a stiff gel called Kenke in Ghana or agidi (or

<u>eko</u>) in Nigeria, by heating the porridges to sufficiently high temperatures and in a sufficiently high concentration. Sorghum, millets and maize can be used. Recently, soybeans have been added to <u>ogi</u> to yield <u>soy-ogi</u>, produced on a pilot plant scale in Nigeria as a weaning food. The microorganisms involved in the fermentations are <u>Lactobacillus plantarum</u> and yeasts.

<u>Mahewu</u> or <u>mogou</u> is produced on an industrial scale in South Africa by inoculation of <u>Lactobacillus</u> <u>delbruckii</u> into autoclaved maize meal, fermenting the mixture for 8-12 hours and spray-drying the slurry. To consume, the dry powder is reconstituted in cold water. It can be fortified with vitamins and yeast extract (Schweigart and Fellingham, 1963 in Okafor, 1980, ref.24).

(b) Alcoholic. These are widely prepared from malted cereals. <u>Burukutu</u> or pito or their equivalents are usually prepared from sorghum. The inoculum is obtained from the previous brew; the organisms being <u>Lactobacillus</u> spp., <u>Geotrichum candidum</u> and Saccharomyces cerevisiae.

Industrial-scale production of pito in Ghana did not last. The production of a heavier beer in Kenya, called <u>Chibuku beer</u>, has apparently met with success. It is made from maize and millet.

Various beers are produced in Zaire, Malawi, Zambia and Tanzania, using maize, sorghum and millet, sometimes with cassava being added. In South Africa, Kaffir beer is produced from sorghum on an industrial scale in two fermentations. In the first stage, lactic acid bacteria derived from the grains produce lactic acid then in the second, alcohol is produced by inoculating with Saccharomyces cerevisiae.

## TABLE 11

Kenya: estimated utilization of whole and sifted maize meal (1978)
 (ref.25)

Non-fermented:	"ugali" stiff paste	81-84%
Fermented :	"ugji" gruel "busaa" opaque beer	10-12% 6-7 % (8 x 10 <sup>5</sup> tonnes/year)

4. Palm-wines (see Table 10)

Palm wines are produced by natural fermentations from the clear, sugary saps of various palms. After collection, the sap turns white from the growth of bacteria and yeasts which are contaminants from the air, tapping utensils and the tree. The counts can easily reach  $10^8-10^9$  per mL. The wine usually turns sour within a short period due to the acid produced by the microorganisms. In general, lactic acid bacteria and yeasts are the initial organisms, followed by acetic acid bacteria. Palm-wines are preserved by pasteurization and marketed on pilot scale by two institutions in Nigeria.

The Federal Institute of Industrial Research at Oshodi near Lagos, pasteurizes its palm wine for 30 minutes and this reduces the viable count to nil and the bottled product can be stored indefinitely. Palm-wine from Cameroon was found to have a pH of 3.9, total acidity of 2.49 g/L and was free of pathogens (ref.28). Pilot plants with capacities of 100, 200 and 500 liters per day are available.

Cameroon is a country where palm wine is a major portion of alcoholic beverages: in 1969/70, 280 of the 405 million liters of alcohol consumed was palm wine.

5. <u>Miscellaneous alcoholic fermented foods</u> (see Table 10) Relishes and stew condiments which result from fermentation include <u>iru</u> (<u>dawadawa</u>) produced from the seeds of <u>Parkia</u> sp. or, more recently, soybean. Others include <u>ugba</u> (or <u>ukpaka</u>) from the seeds of the oil-bean or <u>ogili</u> from the castor-oil plant. Alcoholic beverages are also prepared from fruits of wild plants, honey, sugar-cane, banana, and pineapples in various parts of east and central Africa. Kola is processed into wine on an industrial scale in Ibadan, Nigeria.

Although microorganisms have been used in preparation of African foods and beverages, it can be seen from the above discussion that it is only in a few processes that microbes have been identified and are used on an industrial scale to produce the foods or beverages in question. Although local tastes and preferences will suffer as a consequence, it is nonetheless desirable to encourage the adoption of the scheme proposed by Okafor (see Table 10, footnotes) to accelerate the use of scientific principles in the production of these foods and beverages to give the same quality every time, while at the same time making it possible to industrialize the processes. Training is required, of microbiologists to characterize the responsible organisms, and of engineers to work hand-in-hand with the microbiologists.

#### FOOD CROP PRODUCTION

Africa's poor agricultural performance is a major reason for it's poor general development (ref.29). African agricultural output fell from a yearly growth rate of 2.7 per cent between 1960 and 1970 to 1.3 per cent in the seventies. But because of the high population growth this meant that output per capita, which had grown at 0.2 per cent in the sixties, actually fell, by 1.4 per cent a year, between 1970 and 1980. This situation is not likely to improve; indeed, it has been forecast to worsen in the next two decades. This is actually not surprising in view of the fact that in the period 1980-2000, Africa will invest the least per head of agricultural population and per unit of arable land, among all developing countries (Table 12). Some other factors contributing to the food crisis in Africa are improved health and decreased infant mortality in the population, unpredictable climatic conditions, the lack of well-trained personnel and unsound agricultural policies of several of the governments.

# TABLE 12

Projected gross agricultural investment (cumulative) 1980-2000 in relation to agricultural population and arable land in 1975 . (ref.30).

	US \$ per head of agricultural pop.	US \$ per ha arable land
Developing countries (90)	1320	2160
Africa	700	890
Far East	930	2600
Latin America	4206	2930
Near East	1790	2190
Low-income countries	575	1880

Food production must be intensified if this gloomy forecast for Africa is to change. The microbiologist must make available his expertise and participate fully in the struggle to increase food output. There are three key areas where the technological expertise of the microbiologist will be beneficial: (a) microbiological control of plant pests and diseases; (b) the use of mycorrhizal fungi in phosphate nutrition of plants, and (c) biological nitrogen fixation.

#### Control of insect pests and diseases

Microbiological control of pests and diseases may be carried out directly by using microorganisms or indirectly through the use of standard plant breeding techniques or pesticides. Both approaches require an understanding of the microorganisms involved in crop destruction, but the second approach is easier to tackle, and should quite rightly receive the major emphasis that it is receiving now. Microbial pathogens of insect pest (bioinsecticides) may come from the over 100 species of bacteria, over 700 species of viruses, over 750 fungi and over 300 protozoans; all hold promise (ref.15). ICIPE (ref.31) in Kenya has a young but very active research program on microbial pathogens of the sorghum shootfly and maize stem borer (ICIPE, 1979 & 1980). Clearly, there is room for collaborative work with institutions in the developed countries.

### Mycorrhizal fungi

Next to nitrogen, phosphorus is the most important plant nutrient; phosphorus deficiency is widespread in tropical soils. Mycorrhizal fungi, both ecto- and endomycorrhizal fungi, are important in the establishment of trees and in the phosphate nutrition of annual crops. Work on extomycorrhizae is underway in Senegal, Ghana and Nigeria, where they have been successfully employed in afforestation, particularly in the establishment of exotic species.

The vesicular-arbuscular (VA) mycorrhizal fungi have received significant emphasis in Nigeria, Ghana, Egypt, Senegal and Upper Volta. The major species in Nigerian soils are <u>Glomus</u>, <u>Gigaspora</u>, <u>Acaulo-</u> <u>spora</u> and <u>Sclerocystis</u> (ref.32 & 33). Results of field trials have shown that VA mycorrhizal fungi do significantly increase cowpea

# TABLE 13

Nitrogen fixing systems involving microorganisms and the quantities of nitrogen they fix. (Assembled from various sources by Ayanaba, 1982 (ref.39)).

	System	Microorganism	Hosts	Range of N fixed (kg/ha/yr)
Ā.	Symbiotic			
	1. Rhizobium/Legume	Rhizobium	About 100 plant species in the 13,000 known	40-400
		(bacteria)	species of Leguminosae	
	2. Frankia/Non-legume	Frankia	Numerous non-leguminous	
		(actinomycete)	trees and shrubs, e.g. <u>Alnus</u> , <u>Myrica</u>	9-362
	3. <u>Anabaena</u> / <u>Azolla</u>	Anabaena azollae (alga)	Aquatic fern of the <u>Azolla</u> species	100-150
в.	Asymbiotic			
	1. Blue-green algae	Several algal genera, e.g.: <u>Anabaena, Aulosira</u> , <u>Gloeotrichia, Cylindrospermum</u> <u>Nostoc</u>	None	30-100
	2. Free-living bacteria	<u>Clostridium</u> , <u>Klebsiella</u> , <u>Azotobacter</u> , <u>Beijerinckia</u> , <u>Azospirillum</u> , <u>Rhodospirillum</u>	None	1-100

and maize grain yields (ref.34 & 35). The major short-coming to the widescale use of the fungi has been the inability to massculture them <u>in vitro</u>. This may soon be overcome, however, if the current efforts at the University of Florida, USA are successfull (ref.36). Meanwhile, there is merit to the philosophy of ensuring that improved, high-yielding crop varieties do not lose the affinity for efficient indigenous fungi (ref.37).

#### Biological nitrogen fixation

Nitrogen is a key nutrient for plant production. The nitrogen supplied from industrial fixation has grown precipitously since 1905, from 400,000 metric tons to over 50 million metric tons in 1980. The five forms of biological nitrogen fixation (BNF) in 1980 contributed 90 million metric tons, worth \$40-50 billion (ref.38). It is projected that by the year 2000, if we continue to rely on chemical fertilizers for food production, the world demand would quadruple, requiring \$40-50 billion. This would require 500 new additional large-scale ammonia plants, all requiring natural gas!

In 1980, Africa produced 167,000 tons of nitrogenous fertilizers whereas the estimated consumption was 494,000 tons (ref.30). This trend is not likely to change, given the current global economic climate. Biological nitrogen fixation (BNF), as a complementary source of nitrogen, assumes more significance, given Africa's food crisis.

The five forms of BNF and the annual rates of nitrogen contributed are shown in Table 13. These will be discussed in turn.

#### A. Rhizobium/Legume Symbiosis

The <u>Rhizobium</u>/Legume symbiosis is easily the most important form of BNF for Africa to exploit because the technology for enhancing fixation through this system is well known. Several developed countries already use the technology routinely. For indigenous legumes, improvement in mineral nutrition and general plant growth will bring about a use of indigenous soil rhizobia. Where there is lack of the requisite strains of bacteria, then introduction of these at the time of sowing is easily accomplished. Some of the major institutions and countries where <u>Rhizobium</u>/ Legume works occurs are given below.

- 1. International Research Institution & Organizations.
- a) The International Institute of Tropical Agriculture, Ibadan, Nigeria.

Work here is concentrated on grain legumes such as cowpeas and soybeans, on tree legumes such as <u>Sesbania</u>, <u>Leucaena</u>, and <u>Tephrosia</u>, and on live mulches such as <u>Psophocarpus palustris</u>. The emphasis on microbiological research has been on strain selection, competitiveness and persistence studies, and inoculant production. Rhizobia have been identified for use in legume cultivation in several African countries (Table 14), and the nitrogen fixation levels in cowpea and soybean have been determined to be adequate for good crop yields (Table 15). Facilities for research, graduate and group training are excellent. A large, diverse collection of over 700 rhizobia are held there.

b) ORSTOM, Dakar, Senegal.

The laboratories of the Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM) are undoubtedly the best equipped and staffed in Africa. <u>Rhizobium</u>/Legume research here is on grain legumes (cowpeas, soybeans, peanut), <u>Sesbania rostrata</u>, and <u>Acacia</u> spp. From the most basic to applied research is conducted here; the work on <u>S</u>. <u>rostrata</u> appears to be most promising. Post-graduate training occurs here although, unfortunately the numbers handled are few.

c) Nairobi MIRCEN, University of Nairobi, Kenya

The Microbiological Resource Center (MIRCEN) at the University of Nairobi, is a <u>Rhizobium</u> MIRCEN. (The other MIRCEN in Africa is located in Cairo; it is a Biotechnology MIRCEN). The objectives of the Nairobi MIRCEN are (MIRCEN NEWS, 1982):

- i. To determine conditions for legume inoculation;
- ii. Maintenance and distribution of Rhizobium;
- iii. Identify alternative, suitable inoculant carriers;
  - iv. Assess fates of rhizobia in soils;
  - v. To evaluate new legume introductions;
  - vi. Training of scientific and technical manpower; and
- vii. To disseminate research findings and information on inoculant technology.

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#### TABLE 14

The effect of N fertilization o Rhizobium japonicum inoculation on the grain yield (T/ha) of soybean cv. TGm 80 at seven locations in Africa.

	5 du -4	Rhizobium inoculant				Location	N ferti-
Location	Uninoc	IRj 2101spc	IRj 2114str	IRj 2111	IRj 2123	inocula- tion, Mean <sup>a</sup>	lizer (90 kg/ha)
Ibadan (Nigeria)	2.66	3.21	2.62	3.57	2.66	3.02	3.16
Onne (Nigeria)	0.36	1.51	1.29	1.74	1.16	1.42	0.81
Bouake (I. Coast)	2.69	2.43	2.20	2.93	2.93	2.62	3.44
Ntwapa (Kenya)	2.33	3.27	4.26	3.85	2.86	. 3.56	2.16
Homa Bay (Kenya)	2.52	2.54	3.00	3.51	3.34	3.10	3.35
Kabete (Kenya)	0.37	0.45	0.23	0.39	0.38	0.36	0.30
Sefa (Senegal)	0.71	0.75	0.77	1.73	1.29	1.14	2.22
Mean ± S.D.	1.66	2.20	2.05	2.53	2.09	2.17	2.20
	±1.12	±1.14	±1.39	±1.28	±1.13	±1.20	±1.25

<sup>a</sup>Mean yield due to inoculation with the four strains at the indicated location

#### TABLE 15

Effects of seed inoculation and  ${}^{15}N$  fertilization on biological nitrogen fixation in cowpea and soybean grown on an Alfisol (Eaglesham <u>et al.</u>, unpublished)\*

Cultivar	<sup>15</sup> N Rate kg/ha	N fixed kg/ha	Seed yield kg/ha
Cowpea cv. ER-1	0	n. <del></del>	1180a
	25	46.9	1430a
	100	25.9	1480a
cv. TVu 1190	0	6- <del>11</del>	180a
	25	97.2	1090b
	100	44.0	1190b
cv. Ife Brown	0	-	1330a
	25	85.7	1570a
	100	40.4	1330a
cv. TVu 4552	0	-	1390ab
	25	51.4	1150a
	100	23.1	1490b
Soybean cv. n59-5253	0	-	4120a
	25	188.3	3500a
	100	93.5	3450a
Soybean cv. Williams	0	_	2490a
	25	125.2	2190a
	100	61.1	2180a

\*LSD Value (5%) of N fixation is 33.4; for seed yield, numbers followed by the same letters are not significantly different at the 5% level, within cultivars.

As a regional unit depending on funds from international sources, there is much potential for the MIRCEN. Thus, the Nairobi MIRCEN has made significant strides since its inception. Some of the major accomplishments of the MIRCEN have included (ref.20):

- i. Traning and regional cooperation. It has conducted several short-term training courses for groups and post-graduate degree training; it has coordinated research on BNF in East Africa.
- ii. It has produced a Newsletter twice each year to inform <u>Rhizobium</u> workers in the region. The demise of the Australian-based <u>Rhizobium</u> Newsletter could create a new role for the Nairobi MIRCEN Newsletter.
- iii. Inoculant production for legume production at the farm level.
- d) U.N. Food and Agricultural Organization Through its fertilizer program and some collaborators, FAO is conducting field trials to assess the benefits of <u>Rhizobium</u> inoculation in several countries (ref.30). The trials are currently in progress.

2. National Research Institutions

BNF technology has been applied in some African countries; in all except the Ivory Coast, the inoculants are produced locally (Table 16). Prices of 150-200 g packets of inoculants do not exceed \$3-4. Most of the inoculants used in Africa are used on soybean, an introduced legume that can show up to 150% yield increases at times (ref.40,41,42).

B. Other symbiotic associations

Research is underway at ORSTOM, in Senegal and in several institutions in Egypt on the <u>Frankia</u>/non-legume and <u>Azolla/Anabaena</u> <u>azollae</u> associations. Unlike in Asia where <u>Azolla</u> has been exploited for agricultural production, the situation is different in Africa. The excellent potential of <u>Azolla</u> in wetland rice cultivation has been shown in Senegal. There, laboratory results showed that 55 kg N/ha/year can be realized from the association; this is nearly half of the quantity of nitrogen required to produce a crop of rice (ref.43).

#### TABLE 16

<u>Rhizobium</u> inoculant production for research and/or legume production in Africa.

Country	Legumes inoculat	ed	Carrier	Rhizobium culture collection
Egypt*	Soybean		Peat, coal	
Ivory Coast	Soybean		Peat	No
Nigeria	Soybean,	Cowpea	Peat	Yes
Kenya	Soybean,	Phaseolus	Filter mud	Yes
Zambia*	Soybean		Peat	No
Malawi*	Soybean,	Peanut	Peat	Yes
Zimbabwe*	Soybean,	Peanut	Bag-silo	?
Senegal	Soybean		Peat + Clay	
South Africa*	Soybean,	forage	Peat, coal	

\* Countries where inoculants are produced in local factories for crop production.

# C. Free-living nitrogen fixers

In Egypt, algae are routinely multiplied and used to inoculate rice fields with beneficial results. El-Haddad (1982, ref.44) reviewed work on blue-green algae in paddy soils, including African work. Pure cultures of <u>Tolypothrix</u> tenuis and <u>Nostoc</u> spp. are employed.

Inhabitants along the shores of Lake Chad are reported to have used <u>Spirulina</u> as food for a long time. The technological level of this industry remains primitive.

At the National Research Center, Cairo, work is in progress to mass-culture <u>Chlorella</u> <u>vulgaris</u> and <u>Scenedesmus</u> <u>acutus</u>, using outdoor semi-pilot units (ref.45).

Some research work on free-living bacterial fixers such as <u>Azotobacter</u>, <u>Beijerinkckia</u> and <u>Azospirillum</u> occurs in several African universities and research stations, but the applicability of this kind of work towards the solution of the problem immediately at hand is doubtful.

#### Support for BNF work

Our best prospect for supplying the increasing amounts of nitrogen required to meet the food production levels up to the year 2000 will remain the traditional methods of searching for freaks or variants among natures own population. The technology for utilizing rhizobia in agriculture has been successfully exploited in the developed countries, particularly Australia, New Zealand and the United States. Equally competent exploitation in Africa has been hampered by several factors, including lack of support by governments, lack of training personnel and absence of suitable laboratories for inoculant production. But this is changing.

Support for BNF work has been provided largely by international organizations and non-governmental organizations. The United Nations Development Programme (UNDP) is a major funder. The role of the U.N. Environment Programme (UNEP) and of UNESCO in establishing the MIRCENS, and of the latter in providing training fellowships are steps in the right direction. Several governments support BNF work through bilateral agreements. An inoculant production plant is required in W. Africa. Current moves to set up a body to promote BNF work in Africa also requires some assistance to get off the ground.

# DEVELOPING BIOTECHNOLOGY IN AFRICA

As long ago as 1974 (ref.46) the following areas were considered extremely important in African development:

- Biological leaching of minerals, e.g. the copper plant in Chilanga, Zambia;
- 2. Production of edible proteins from petroleum;
- 3. Chlorella for milk substitute;
- 4. Nitrogen fixation by enzymes;
- Genetic manipulation to control diseases and produce selective plant varieties, and
- 6. Non-pollution pesticides, e.g. bioinsecticides.

These are sill very important and will continue to be so. To this list must now be added production of alcohol and biogas to meet energy needs. In some of these, particularly the ones which have been in Africa for long, the processes are still poorly understood or developed. In the newer processes that have been introduced, pilot plants or industrial production is already in progress.

The promotion of biotechnology in Africa must be the number one objective. Education of policy makers and training of microbiologists and engineers must receive priority. Local talent must be utilized. All these are necessary for the technological advancement of Africa. The thrusts must be both at the national and regional levels.

At the national level, the recent establishment of the Appropriate Technology Advisory Committee (Atac) in Kenya (ref.47) is worthy of emulation by other African countries. Atac was set up to fund people whose ideas can be developed into simple technologies at the village level. This will stimulate innovation. The Appropriate Technology Institute in Washington has already indicated its support for this kind of activity by providing \$500,000. Atac will begin by approving plans by only 10 innovators, each with \$2,500, along with very stringent measures.

Indeed, what is happening in Kenya is in accord with the noble objectives expressed by African heads of state (ref.48), that a new strategy for science and technology base has to be evolved for the third United Nations Development Decade (1980-1989). Two of the items on the new strategy are pertinent here. First, each Member state of the Organization of African Unity (OAU) is to establish a National Center for Science and Technology for Development, which Center should formulate policies, assist indigenous technologies, identify the country's needs and assist in negotiating the transfer of technology, from a position of strength. Secondly, in order to mobilize the funds necessary for the successful development of science and technology, each member state is to aim, at the domestic level, to gradually reach a point where they devote 1% of the GDP for development within the decade. The ways of securing funds to realize these objectives are clearly spelt out in the Lagos Plan (ref.48). But chances of accomplishing anything in the decade are very slim, in many countries, given the current global economic crisis. The route that Kenya has taken must be an example.
At the regional level, it is assumed that the Dakar-based African Regional Center for Technology (ARCT) will take a leading role to promote, fund and coordinate technological development and transfer in Africa (ref.49). Biotechnology would naturally be included in the activities, particularly, in areas as timely and important as gasohol and biogas production. For the moment, however, this young institution, which was only established in January 1980, has the following activities planned for five years 1982-86:

- 1. Development of the Center's capabilities;
- 2. Promotion of indigenous and other technologies in the food industry;
- 3. Promotion of advisory services in technological consultancy, training, and information and documentation.

The ARCT derives its funding from contributions of member states of the Organization of African Unity (OAU), from fees charged for services rendered and from assistance, aid, loans, etc. from governments and international organizations. The ARCT would therefore be an appropriate organization to channel requests on and support for biotechnology in the African region.

Also at the regional level, professional associations should come together to promote biotechnology. The African Network of Microbiological Societies, with current headquarters in Nigeria, aims to do just that. Also, a group of individuals interested in BNF met recently at the IITA in Nigeria, to draw up plans for promoting the use of BNF for food crop production. Both of these are young, needing all the encouragement, advise and support that can be provided.

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#### REFERENCES

- 1. Stoker, J.A. 1979. West African Technical Review. September, pp. 62-63.
- Anon. 1982. New refuse collection system in Lagos, Sunday Con-cord, August 15 page 1.
- 3. Anon, 1983. West Africa. October 4.
- Sanni, S.O., 1980. in GIAM VI Proceedings p.227 (see ref.37).
   Anon. 1974. Nightsoil pollutes Lagos Waters. New Nigerian, Thursday 15 August, p.16.

- 6. Food and Agriculture Organization. 1973. Agricultural Services
- Bulletin, No.21, FAO, Rome.7. El-Nawawy, A.S. 1978. Waste Utilization for SCP production in the ARE. GIAM V State of the Art, Stanton and Da Silva, eds., UNEP/UNESCO/ICRO Panel, page 139.
- 8. Anon, 1982. Power alcohol in Tanzania. Africa Research Bulletin 19 (4): 6423B.
- 9. Anon, 1981. Ethanol in Malawi. Africa Research Bulletin 81 (1): 5819B.
- Anon, 1982. Alcohol plant in Mauritius. Africa Research Bulle-tin. 19(5):6461A.
- 11. Anon, 1982. Ethanol plant in Zambia. Africa Research Bulletin 19(6): 6501.
- 12. Anon, 1982. Alcohol plant in Kenya. Africa Now. No.178, Julyp.51.
- 13. Anon, 1979. Fuel from cassava. West African Technical Review. September. pp. 57-61.
- 14. Berkovitch, I. 1979. West African Technical Review. October, pp. 91-93.
- 15. National Academy of Sciences. 1979. Microbial processes: promising technologies for developing countries. NAS.
- Reynolds, G.F. 1975. Appropriate Technology 2 (2): 11-13.
   Odeyemi, O. 1980. In GIAM VI Proceedings p.245. (See ref.37). 18. Lyamchai, A.A. and Bhatia, B.S. 1980. In GIAM VI Proceedings p.272 (see ref.37).
- 19. Langley, P. 1978. Environnement Africain. November, no.21.
- 20. MIRCEN News. 1982.
- 21. Hols, Ir. P. 1981. Biogas. AT-News 2(1): 3-8.
- 22. El-Halwagi, M.M., Dayem, A.A. and Hamad, M.A. 1982. Biogas technology for rural areas of Egypt. First OAY/STRC Bio-Fertilizer Conference, Cairo. March.
- 23. Santerre, M.T. and Smith, K.R. 1982. World Development. 10(3): 239-261.
- 24. Okafor, N. 1980. In GIAM VI Proceedings. p.61 (See ref.37).
- 25. Nout, M.J.R. 1980. In GIAM VI Proceedings p.169. (See ref.37).
- Abd-El-Malek, Y. 1978. In GIAM V. UNEP/UNESCO/ICRO Panel.
   United Nations. 1978. Technologies for developing countries.
- Fyot, R. 1973. Techniquies et Development. Juillet-Aout. No.8.
   West Africa. 1982. August 23. p.2147.
- 30. Ceres. 1980. FAO's program on BNF. 13(1): 4-5 and Nitrogen Production in Africa. FAO Ceres 13(4):5-7.
- 31. International Centre for Insect Physiology and Ecology (ICIPE). 1979 and 1980 Annual Reports.
- 32. International Institute of Tropical Agriculture (IITA). 1976. Annual Report. pp.80-81.
- 33. Sanni, S.O. 1976. New Phytologist 77: 667-671.
- 34. Islam, R. and Ayanaba, A. 1981. Plant and Soil. 61: 341-350.
- 35. Islam, R. and Ayanaba, A. 1981. Plant and Soil. 63: 505-509.
- 36. Siqueira, J.O., Hubbell, D.H. and Schenck, N.C. 1982. In program of Abstracts of the 13 Int. Congr. Microbiol. August. 37. Hahn, S.K., Kang, B.T. and Ayanaba, A. 1980. GIAM IV Procee-
- dings, Emejuaiwe, Ogunbi and Sanni, eds. Academic Press, London. 38. Wittwer, S.H. 1977. In: Genetic Engineering for Nitrogen Fixa-
- tion. Plenum Press, pp.515-519.
- 39. Ayanaba, A. 1982. Impact of Science on Society 32(2): 179-187.
- 40. Rao, V.R., Ayanaba, A., Eaglesham, A.R.J. and Kueneman, E.A. 1980. In GIAM VI Proceedings. p.153.
- 41. Bromfield, E.S.P. and Ayanaba, A. 1980. Plant and Soil. 54: 95-106.

- 42. Kang, B.T. 1975. Expt. Agric. 11:23-31.
- Ceres. 1981. Azolla potential studies for use in African rice fields. FAO Ceres 14(2): 10.
- 44. El-Haddad, M.M. 1982. The blue green algae in paddy soils. First OAU/STRC Inter-African Conference on Bio-fertilizers. Cairo. March.
- 45. Fouly, E., Abdalla, F.E., El-Baz, F.K. and Fawzi, A.F.A. 1982. Mass production of microalgae in the national Research Center. First OAU/STR Conference, Cairo. March.
- 46. UNESCO, 1974. Selected Technologies for African Development, Paris.
- 47. Kyalo, R.C.K. 1982. Kenya rewards new technology. April., no. 18 p.32.
- Lagos Plan of Action for the Organization of African Unity. 1980. Chapter V. Science and Technology.

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49. Anon. 1978. Regional Center to strengthen Africa's technological capabilities. Rural Progress. 1: 16-19. BIOTECHNOLOGY IN DEVELOPING COUNTRIES: THE SITUATION IN LATIN AMERICA

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### ABSTRACT

Is there a role for biotechnology in fostering technical, economical and social development in Latin American countries? If so, what type of role? What is the required infrastructure needed for biotechnology to function properly and produce short and long term results? Is biotechnology understood by decision makers, entrepreneurs and last but not least, by scientists and engineers themselves? What are the main obstacles that could hinder biotechnological developments? What type of research and development is needed? What type of agro-industrial development biotechnology would favor? All of the above are key questions relevant to a discussion of biotechnology in developing countries. Specific answers are as yet not available. Points of view and positions are emerging which are useful only in as much as they provide a basis for discussion.

This presentation is divided in two parts. The first one will be a set of opinions of general nature, which, responding to the above questions, will lead to the following specific conclusions: a) biotechnology is extremely important for the Latin American region, b) the human resources needed must be trained and ought to be incorporated in already functioning productive research groups, c) research creativity is a sine qua non condition, and d) exchange programs within the region and with other regions should be established.

The second part of the paper is more specific and summarizes results of innovative research pertaining to Central American conditions. Topics include the utilisation of agroindustrial wastes for food and feed production and biofuels from renewable resources.

# LATIN AMERICA AND BIOTECHNOLOGY

Latin America has about 8% of the world population and although, due to its history and cultural heritage, represents a relatively homogeneous ethnic group, local conditions exist in each subregion and in each country which influence greatly the type of social, cultural and economic development sought. Nevertheless, the proper use of technology has spearheaded an industrial development, which in turn, has caused a dynamic trade within the region, mainly in manufactured goods.

The region, as other developing nations, is a net importer of technology and technological products and an exporter of agricultural, mineral (fossil fuel) and marine commodities. There is some support at the national, subregional and regional level for research and development activities. Their output, however, is still low as well as their use by private entrepreneurs or development corporations. Scientific endeavors within the region with a practical objective do not exist at the level that they ought to.

The present situation of biotechnology is no different. Its future large scale application to solve crisis-oriented problems (with the new incentive provided by genetic engineering) in food and feed, alternative forms of energy, chemical feedstock supply, health care problems and environmental concerns has been largely acknowledged by Latin American bioscientists. Their priorities however do differ from those in developed countries or those from other developing regions. However, it is also a fact, that biotechnological dependence will be just a matter of time. Specially when one considers the human and physical resources already committed in industrialized countries for this task. There is practically no way to match it.

Some regional comments, in part from information and suggestions received from: D.A.S. Callieri, G. von Ellenrieder, and F. Siñeriz (Argentina); W. Borzani and T.J. Barreto de Menezes (Brasil); J.C. Gentina and the professors of Escuela de Ingenieria Bioquimica, Valparaiso (Chile); R. Quintero and A. Cabello (Mexico); F. Castillo and V. Carrizales (Venezuela) and J.A. Cipolina (Uruguay), about a) the understanding of biotechnology, b) the training of biotechnologists, c) the type of research done and its transfer to potential users and d) the type of agro-industrial development that biotechnology could foster, follow.

a) Understanding biotechnology: although bioscientists and bioengineers will probably argue for a proper definition for some years to come, the important point here is not the definition <u>per</u> se but its description to nonscientists. Although there has been a good deal written of the new biotechnology, the publications have not reached as yet the proper audience in Latin America. There is only a superficial knowledge in the public sector of the real potential that biotechnology has for the region. Brasil, Chile, and Mexico seem to be betteroff and in them, the National Science and Technology Centers or their equivalent, have already discussed and designed a National Biotechnology Program. Local industry in general is not aware of the possibilities with the exception of large multinationals or subsidiaries. However the opinion of some, is that biotechnology will improve the interaction between the scientist with the private local entrepreneur. In the long run, collaborative research with industry itself is even contemplated.

b) <u>Training of biotechnologists</u>: in some countries (Brasil, Chile, Mexico, Venezuela) there are Graduate Programs in Biotechnology. In others, chemical engineers, biochemists and microbiologists usually take introductory courses during undergraduate training and in some cases applied research projects are done in technological institutes. As a matter of fact, a general consensus exists that the main obstacle for a proper biotechnological development is the lack of advanced level scientists with the proper motivation.

c) <u>Research done</u>: research topics usually are chosen in agreement with and within priority areas of the national development plans. Otherwise it is almost impossible to obtain grants. A great deal of research is repetitive work (very few admit it) but usually this is done before more ambitious and original work is carried out. Usually, however, before this phase is reached, funds are no longer available. Even so, few research activities produce results which are internationally recognized. Research results are usually published in local or regional journals (in some cases with a long lag in between) that have a rather restricted circulation. Technological newsletters of wide distribution are usually the exception and not the rule.

d) Agroindustrial development: nearly 60% of the tropical rain forests of the world are located in Latin America. A very large

number of species of plants, algae, fungi, microalgae and bacteria needs to be screened with respect to their potential. It is very unlikely that organisms of potential interest are to be encountered only in the natural ecosystems of developed countries. The region also produces 66% of the world coffee crop, 53% of the bananas and, 44% of the world sugarcane harvest. All these factors imply a tremendous amount of present and potential biomass resources. A country in the region (Brasil) has started a unique ethanol-fuel program that has stimulated agroindustrial development.

The points of view and positions described are useful in providing the following conclusions:

- a) Due to available natural resources and the emerging human resources biotechnology is an extremely important option for a much broader agroindustrial development.
- b) Technologies or processes that generate multiple products from biomass should be emphasized. Modeling techniques, including engineering, economic analysis and energy and material balance, as well as macro-economic aspects (for example, rural or centralized, mini or maxi-plants), should be used for the evaluation of options.
- c) The region needs to invest in order to create biotechnological developments. The best investment to be made is in human resources training at an advanced level, with a specific effort being made towards creativity and inventiveness.
- d) Appropriate biotechnology must be developed of such quality that it will compete with any available in the market. This requires research of the highest level and optimum research management.
- e) Scientific exchange programs within the region and with other regions (industrialized or developing) should be encouraged. International and bilateral scientific and technical support programs should complement such endeavors by providing specific research grants to advanced training programs and to networks of research institutions (for example the MIRCEN concept).
- f) Genetic engineering should be viewed as a powerful tool to be employed when required and only if it is the best option available.

### BIOTECHNOLOGY IN WASHED COFFEE PROCESSING

Among natural commodities in international trade, coffee usually ranks second only to petroleum in dollar value, accounting for approximately US \$ 12 billion per year. All of 50 exporting countries rely upon it as a major source of foreign exchange although it is the raw dried bean which is traded and its final processing is done in the industrialized countries where billions have been invested in equipment. Some 40 million people in the rural areas depend heavily on coffee for their livelyhood and all over the world unaccounted millions drink it by the potful.

Almost half of the world coffee production is processed by the socalled wet method (ref.1) in which the skin or pulp is removed mechanically, then the mucilage is fermented by natural microflora (ref.2) and the bean is washed, dried and dehulled. Three byproducts are generated during processing: the pulp, the mucilage and the hulls in ratios of 40, 20 and 3.4 per 100 weight of fresh fruit; only in tropical America seven million tons of byproducts are produced each year (ref.3).

In common with many other agroindustrial activities worldwide coffee processing plants have been facing two major problems: i) waste disposal, and ii) energy for drying. Roughly, the different dryer designs in operation require from 4-9 GJ/ton of dried dehulled coffee produced and in many systems fossil fuels are currently being employed. Our present research strategy has evolved from an analysis of alternatives (ref.4,5) where combined byproduct utilization with fuel generation are the specific objectives pursued through the synthesis of multipurpose processes.

Coffee pulp is discharged as a wet solid material which is practically impossible to store and keep without natural biodegradation, hence it is the cause of pollution problems through leaching and bad odours.

Anaerobic solid state digestion with biogas generation is one of the possible waste treatment methods available. Laboratory tests were done with fresh and aerobically pre-composted pulps (ref.6).

### TABLE 1

Substrate	Tempera- ture (°C)	Retention time (days)	Volumetric production (vol/vol day)	%CH4	pH
Fresh pulp	35 35	60 40	1.30	60 48	7.5
Pre composted pulp	35 35	60 40	0.20 0.02	72 80	7.5

Biogas production from fresh and composted coffee pulp

As seen in Table 1 high rentention times, typical of solid state biodegradations, were required. Fresh pulp was a much more biodegradable substrate than the pre-composted material, which has been liberated from soluble matter during the aerobic thermophilic composting. Pulp treatments were explored in order to separate the soluble compounds from the insoluble matrix. To this effect pilot trials were carried out with a batch hydraulic press employing fresh coffee pulp or pretreated by the addition of commercial pectic enzymes or by heating with live steam or both (ref.7). By proper combination, juice yields were close to 60% (w/w fresh pulp) extracting with it about 35% of the total solids present. The juice had about 75% of the total hot water extractable sugars present in fresh coffee pulp, all the polyphenols, about 15% of the nitrogen and 30% of the caffeine. The pressed solids had a final moisture around 70%. Pressing trials were also done employing a continuous screwpress. Juice yields without any pulp pretreatment were around 42% (w/w fresh pulp) operated at a pressure of 0.44 to 0.51 MPa (50-60 psig) and about 1000 kg fresh product per hour. One and two stage biodigestion systems have been tested in the laboratory employing coffee pulp juice and with loading rates ranging from 0.5 to 3 g volatile solids  $1^{-1}$  day<sup>-1</sup> and hydraulic retention times of 5-10 days in the methanogenic stage (ref.8).

In Table 2 operating data at 35°C of the continuous acidogenic stage, at 0.5 day HRT (hydraulic retention time) is given. A high sugar conversion and a lactic to acetic ratio in the product of 2.9 were obtained.

### TABLE 2

Acidogenic stage (0.5 day HRT) in two-stage anaerobic system

محقق می المحقق الم	Daily load (g/L)	Daily discharge (g/L)	Reduction (%)
Volatile solids	55.8	30.2	45.9
Nigrogen	1.0	0.8	20.0
Total sugars	51.0	5.6	89.0
Acetic acid	3.2	4.6	-43.8
Lactic acid	8.4	13.2	-57.1

Figure 1 gives data of the methanogenic second stage with 8 days HRT at 35°C. A stable volumetric daily gas productivity of around 1.3 was obtained. A stable operation was possible at 6 days HRT only if the juice feed was diluted 25% with tap water.

FIGURE 1

METHANOGENIC STAGE: 8 DAY, HRT; 1.84 G/L DAY



In Table 3 operational data are given for the one stage system. Stable operation at 10 days HRT was possible only by diluting drastically the fresh pulp juice. New designs of laboratory digesters are currently being operated, in which biomass has been retained in the methanogenic stage. One of these is what we have called the "sponge" digester", where modules of polyurethane rigid foam are

used as packings for column digesters operated in an up-flow mode.

TABLE 3

Gas productivity and composition, pH ranges in one-stage anaerobic system (10 days HRT)

Juice in feed (%)	Volatile solids loads (g/L)	Gas productivity (vol/vol day)	°CH4	рН
10	0.49	0.74	66	6.0-8.0
20	0.97	1.55	79	7.4-7.7
40	1.95	1.48	80	5.3-6.6

An alternative use of the coffee pulp that has been tested is to use it as substrate for microbial biomass production. The batch growth at 30°C and an initial pH of 3.5 of the following filamentous fungi: <u>Aspergillus oryzae</u>, <u>Trichoderma harzianum</u>, <u>Penicillium crustosum</u> and <u>Gliocadium diliquescens</u> was published (ref.9). Tests have also been carried out with <u>Candida utilis</u> (ref.4). The following biomass production alternative was recommended for scale up: i) batch growth up to 12 to 13 h, ii) whole broth concentrated to syrup consistency, iii) syrup storage, iv) mixed with grains (sorghum) and used in poultry or swine feed, and v) mixed with grasses, straws or pressed coffee pulp, ensiled and fed to ruminants.

Results have been presented before (ref.3) where it has been shown that mycelium storage of <u>T</u>. <u>harzianum</u> in a 62.5° Brix syrup for one month induced lysis and most of the nitrogen had gone into solution. Such technique then might be a novel method of extracting nitrogen and having it more available for animals. Also it was shown that <u>P</u>. <u>crustosum</u> could utilize chorogenic acid and caffeine as source of carbon and nitrogen for growth (ref.3) thus reducing the final concentrations in the residual broth ; these compounds in high concentrations might have deleterious effects on animals.

One alternative for utilization of the pressed coffee pulp is to decompose it aerobically in a pile in order to produce good quality organic fertilizer for use in plant breeding and coffee nursery or seedling operations. Pilot trials were done with induced air flow through piles of fresh pulp and this was decomposed in a period of three to five weeks to a stable product. The average 110 pile temperature increased to values between 60-70 °C during active thermophilic microbial activity, period where the air oxygen content in the pile had decreased to around 2%. The pulp had an initial apparent density of 540 kg m<sup>-3</sup> and it was reduced by 33% after 3.5 weeks. The final product had a total nitrogen and ash contents of 6-7% and 17-23% respectively, and from an acidic pH it had turned slightly alkaline.

Another alternative for the use of the pressed pulp is as substrate for mushroom growth, alone or in mixtures with other lignocellulosic substrates like straws and bagasse. The most interesting point here is that after the fruiting bodies of the mushroom have been collected the residue can be used as an animal feed as usually its nutritional characteristics have been improved. Trials have been completed on the growth of <u>Pleurotus flabellatus</u> (oyster mushroom) ITCCF 1724 from the Indian Agricultural Research Institute, New Delhi, on pressed coffee pulp, alone or in mixtures with wheat straw and citronella bagasse. Yield data are presented in Table 4 where the beneficial effect of mixing substrates is easily seen. Pressed coffee pulp and wheat straw gave the best response.

### TABLE 4

	(weight of fresh mushroom/ weight of dry substrate) 100
1) wheat straw	64.17
2) citronella bagasse	12.99
3) coffee pulp	41.30
4) 1 + 2	82.87
5) 1 + 3	96.08
6) 2 + 3	52.00

Growth of the mushroom Pleurotus flabellatus

## SUGAR CANE TO ETHANOL: THE EX-FERM PROCESS

Ethanol from cane and its byproducts can be produced in sugar milldistillery combinations or in independent units which use as raw material the extracted sucrose-rich cane juice. Most of the recent units built in Brasil still use the batch type fermentors either for dilute molasses or cane juice feed. Some other international companies are offering a continuous fermentation system as a standard unit in their different designs. Less experience exists in the fermentation of sugarcane juice than in that of molasses. In mixed fermentations (juice and molasses) the ethanol yield decreases as the percentage of molasses in the mixture increases. Practically there is no commercial experience in fermenting sweet sorghum in any form, juice or molasses. In some tropical countries virgin molasses (concentrated lime treated juice) are fermented for rum production.

An analysis of the equipment costs of standard designs for independent distilleries has been discussed previously (ref.10). It was shown that more than 50% of the total cost is tied up in equipment for canepreparation/juice extraction and plant utilities. Even higher values have been reported recently for non-fermentation equipment requirements (ref.11). Ethanol production costs in these units has also been analyzed (ref.10) and it has been shown that raw materials and nutrients represent about 70% of the total ethanol production cost, depreciation and interest 24%, and the other 6% incorporates everything else. Of the total energy required for processing about 46% is employed for cane preparation/ juice extraction, from 21 to 39% for ethanol recuperation (according to distillation unit efficiency) and the rest employed in electricity generation for process motors.

Total raw material utilization and conversion into ethanol is then the major objective to look for in order to decrease production costs as it represents the major item in the process cost structures. The amount of sucrose extracted from the cut stalks of cane or sweet sorghum and from the tubers of sugar beets or the inulin from Jerusalem artichokes bears heavily on the final cost of ethanol produced. Whatever fermentable sugar is left in the lignocellulosic residue is burned with it in the steam boilers. Industrial sugar extraction even in highly mechanized and large scale units is only about 95% complete (ref.12) being close to 90% in smaller and less efficient systems, either employing milling or hot water extraction. As a matter of fact, Brasil has developed the concept of the micro or mini distillery, plants with an ethanol production of less than 5000 L per day (ref.13). Sugar extraction in such small milling systems is only 61%. In order to improve this situation, specially for developing countries where small plants will

be more appropriate, a process was described (ref.3) in which sugarcane was cut into chips of proper size, and water and a yeast inoculum added. Then as sugar was extracted from the cane it was also fermented by the yeast. In a subsequent publication (ref.14), the process idea was expanded so as to use again the ethanolic yeast suspension from the previous fermentation by mixing it with a new batch of fresh cane chips. The process was called EX-FERM and thought to be a novel solid-liquid mixed phase fermenation where there was a concurrent <u>extraction and ferm</u>entation of cane sucrose and other sugars into ethanol.

Laboratory scale trials of a two cycle EX-FERM procedure employing 37 strains of <u>Saccharomyces</u> spp. have been reported (ref.15). Two types of cane treatments were used: chips and shredded pith, either fresh or previously air dried (at 60°C) to moisture levels between 0.1 to 3%. The 0.5 to 2.2 cm cane chips were obtained in a pilot wood chippers, yields varied from 87 to 99% of the cane milled, the rest being oversize chunks and undersize powder separated on circular hole screens. The shredded pith was obtained in a pilot Tilby machine (ref.16). The presence of cane solids and other soluble compounds did not inhibit ethanol formation, however it did not enhance it either. Product yields were within accepted values and differences found among yeast strains for either cane chips or shredded pith were not significant.

Preliminary results of laboratory column packed bed fermentors have been also published (ref.17) and also detailed operational data for two and three EX-FERM cycles employing horizontal and vertical packed bed fermentors (ref.18-22). A vertical packed bed pilot unit has been constructed, initially tried as a trickle bed fermentor and actually operated in order to optimize ethanol productivity and to quantify the yeast distribution between the solid matrix and the fermenation broth. A summary of the experimental results is presented in Table 5. It shows the gradual improvement of ethanol productivity as the scale of the fermentor was increased. This was possible by reducing cycle time. Most of the data presented were obtained with previously dried cane chips or shredded pith. Sugar consumption was excellent and although alcohol yields seem low, it is believed that some alcohol was lost in the operation of the packed columns with the external circulation. 114 TABLE 5

Summary of EX-FERM experimental results

5 <u>cale</u> 1)	500 mL flasks	Substrate/Operation Fresh cane chips/first cycle Dried cane chips/second cycle Fresh cane Tilby pith/first cycle Dried cane Tilby pith/second cycle	Yeast <u>Strains</u> 37 18 37 18	Cycle <u>time,h</u> 40 27 40 27	Conc 9 <u>Cs1</u> 7.3 4.0 4.9 3.9	entra /100 Cxi .06 - .08 -	tions mL 2.9* 1.5* 2.0* 1.4*	water to dry solids ratio 11.9 16.0 12.6 16.0	Sugar consump- tion 96 91 97 81	Y <sub>P/S</sub> .41 .40 .43 .44	produc- tivity <u>g/L.h</u> .7 .5 .4 .5
2)	2 L vertical loop packed bed reactor	Dried cane chips/first cycle Dried cane chips/second cycle	12 12	24 24	5.0 5.0	.37 .28	3.6 4.7	11.6 11.9	94 93	.46 .46	.9 .9
3)	700 mL horizontal loop packed bed reactor	Dried cane Tilby pith/first cycle Dried cane Tilby pith/second cycle	12 12	12 12	4.0 2.0	.24 .04	2.8 3.2	17.1 17.1	93 72	.47 .40	1.6 1.0
	30 L vertial loop packed bed reactor	Dried cane chips/first cycle Dried cane chips/second cycle Dried cane chips/first cycle Dried cane chips/second cycle Dried cane chips/first cycle Dried cane chips/second cycle	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	7 8 9 9 12 11	6.3 11.8 5.7 6.8 6.3 7.6	3.0 - 2.0 - 0.5 -	2.5 6.0 2.8 6.0 1.8 4.0	8.5 4.3 6.8 6.8 7.2 5.9	97 98 69	.36 .44 .42	3.6 4.8 3.1 3.4 1.5 1.3

\* Not alcohol produced in each cycle. Csi = theoretical initial sugar

Cxi = initial yeast concentration

Cp = final alcohol concentration

concentration

There is no need to obtain a shredded pith, the cane chips are more suitable for EX-FERM operation and present the best alternative available in terms of ethanol yield from weight of fresh cane as shown in Table 6.

#### TABLE 6

Yields of ethanol from sugarcane employing different technologies

	Base 1000 kg cane	
125	kg 130 l	kg
Solid resid	due Ferm	entable sugars
Traditional technology	↓ Tilby process	<b>↓</b> EX-FERM
87% extraction	93% extraction	98% extraction
efficiency	efficiency	efficiency
0.46 kg ethanol/	0.46 kg ethanol/	0.46 kg ethanol/
kg sugar	kg sugar	kg sugar
52.03 kg ethanol	55.61 kg ethanol	58.6 kg ethanol
99% recovery	99% recovery	98% recovery
efficiency	efficiency	efficiency
65.2 L ethanol	69.69 L ethanol	72.69 L ethanol

Er-el et al. (ref.23) tested the extraction-fermentation of sugarcane segments in a high solids rotating drum fermentor. A very low initial water to dry solids ratio of about 5.67 allowed them to obtain 83 and 92 g ethanol  $1^{-1}$  in 3 and 4. 35 h cycles. The sugar utilized was around 91-94%. Shalita et al. (ref. 24) tested two <u>S. carlbergensis</u> strains from Wadensil, Switzerland, and a <u>Z</u>. <u>mobilis</u> strain CP4 with cane chips previously dried at 62°C for 72 h. With the yeasts they obtained a sugar consumption of 96.4 - 98.5%, an alcohol yield of 0.40-0.47 in a cycle of 72 h at 32°C. With the bacteria the figures were 89.1-95.6 and 0.39-0.52 respectively.

A solid phase extraction-fermentation system developed at SCIRO, Australia for beets has been recently reported (ref.25). Yeast is directly mixed with washed chopped beets. A final ethanol concentration of 95 g  $1^{-1}$  was reported: 95% of the alcohol could be recovered by two successive pressings with a small interstage wash. For comparison only 65% of the sugar was extracted with the same procedure in unfermented chopped beets. Larsen et al. (ref.26) added an equal amount of water to sliced beets and obtained alcohol yields of 0.3 to 0.4 and 0.4 to 0.47 in unheated and previously heated mashes. They also report fermentation data of expressed juice, diffusion juice and of different beet cultivars.

### REFERENCES

- 1 Espinosa, R.; de Cabrera, S.; Maldonado, O.; Rolz, C.; Menchú, J.F. and Aguirre, F. "Protein from waste" <u>Chem. Tech.</u> <u>6</u>: 636 (1976).
- 2 Menchú, J.F. and Rolz, C. "Coffee fermentation technology" <u>Café</u>, <u>Cacao, Thé</u> <u>17</u>: 53 (1973).
- 3 Rolz, C. "Particular problems of solid waste reclamation in developing countries". J. Appl. Chem. Biotechnol. 28: 321 (1978).
- 4 Rolz, C.; Menchú, J.F.; Calzada, F.; de León, R. and García, R. "Biotechnology in washed coffee processing" <u>Process Biochem.17</u> (2) 8 (1982).
- 5 Gracía, R.; Porres, C.; Calzada, F.; Menchú, J.F. and Rolz, C. "Economic evaluation of alternative energy sources for coffee bean drying" in "Food Drying" G. Yaciuk (ed) IDRC-195e, p 94, (1982).
- 6 Calzada, J.F.; de León, O.R.; de Arriola, M.C.; de Micheo, F.; Rolz, C.; de León, R. and Menchú, J.F. "Biogas from coffee pulp" <u>Biotechnol. Letters 3</u>: 713 (1981).
  7 Rolz, C.; Menchú, J.F.; de Arriola, M.C. and de Micheo, F.
- Rolz, C.; Menchú, J.F.; de Arriola, M.C. and de Micheo, F. "Pressing of coffee pulp" Agr. Wastes 2: 207 (1980).
   Calzada, J.F.; de Porres, E.; Cabello, A.; Yurrita, A.; de
- 8 Calzada, J.F.; de Porres, E.; Cabello, A.; Yurrita, A.; de Arriola, M.C.; de Micheo, F.; Rolz, C. and Menchú, J.F. "Biogas production from coffee pulp juice: one and two stage systems" submitted for publication.
- 9 de León, R.; Calzada, F.; Herrera, R. and Rolz, C. "Fungal biomass production from coffee pulp juice" <u>J. Ferment Technol.</u> <u>58</u>: 579 (1982).
- 10 Rolz, C. "A new technology to ferment sugarcane directly: the EX-FERM process" Process Biochem. 15 (6) 2 (1980); Int. Sugar J. 82: 47 (1980)
- 11 Fong, W.S.; Jones, J.L. and Semrau K.T. "Cost of producing ethanol from biomass" <u>Chem. Eng. Prog. 76</u> (9) 39 (1980).
- 12 Palaci, J. "Experience with the Saturne cane diffuser" Int. Sugar J. 75: 267 (1973).
- 13 Hulett, D.J.L. "The development of a microdistillery for fuelalcohol in Brazil" <u>Sugar J.</u> 44 (5) 7 (1981).
  14 Rolz, C.; de Cabrera, S. and García, R. "Ethanol from sugarcane:
- 14 Rolz, C.; de Cabrera, S. and García, R. "Ethanol from sugarcane: EX-FERM concept" <u>Biotechnol. Bioeng. 21</u>: 2347 (1979).
  15 Rolz, C. and de Cabrera, S. "Ethanol from sugarcane: flask ex-
- 15 Rolz, C. and de Cabrera, S. "Ethanol from sugarcane: flask experiments using the EX-FERM technique" <u>Appl. Environ. Microbiol</u>. <u>40</u>: 466 (1980).
- 16 Rolz, C. "Ethanol from sugar crops" Enzyme Microb. Technol. 3: 19 (1981).

- 17 Rolz, C.; de Cabrera, S.; Morales, E.; deArriola, M.C. and de Micheo, F. "The EX-FERM process for ethanol production" Proc. IV
- Int. Symp. Alcohol Fuel Technology 1: 23 (1980). 18 Rolz, C.; de Cabrera, S.; Morales, E. The EX-FERM process" in "Advances in Biotechnology" (Moo-Young, M. and Robinson, C.W.,
- eds) Vol. II, p 113, Pergamon Press, Toronto (1981). 19 Rolz, C.; García, R. and Calzada, F. "Ethanol from sugar crops: developments and strategies" Proc. 2nd World Congress Chem. Eng. 1: 301 (1981).
- 20 de Cabrera, S.; de Arriola, M.C.; Morales, E.; de Micheo, F. and Rolz, C. "EX-FERM ethanol production in packed bed fermentors: a three cycle operation employing chipped sugarcane" Biotechnol. Letters 3: 497 (1981).
- 21 de Cabrera, S.; de Arriola, M.C.; Morales, E.; de Micheo, F. and Rolz, C. "EX-FERM ethanol production using peeled ground sugarcane in packed bed fermentors". J. Ferment. Technol. 60: 77 (1982).
- 22 de Cabrera, S.; de Arriola, M.C.; Morales, E.; de Micheo, F. and Rolz, C. "EX-FERM ethanol production using chipped sugar-cane in packed bed fermentors" Eur. J. Appl. Microbiol. 14: 21 (1982).
- 23 Er-el, Z.; Battat, E.; Schechter, U. and Goldberg, I. "Ethanol production from sugarcane segments in a high solids drum fermentor". Biotechnol. Letters 3: 385 (1981).
- 24 Shalita, Z.P.; White, M.D.; Katz, M.; Zur, M. and Mizrahi, A. "Ethanol production by Z. mobilis CP4 from sugarcane chips". Biotechnol. Letters 3: 729 (1981).
- 25 Kirby, K.D. and Mardon, C.J. "Production of fuel ethanol by
- solid-phase fermentation" Biotechnol. Bioeng. 22: 2425 (1980). 26 Larsen, D.H.; Doney, D.L. and Orien, H.A. "Production of ethyl alcohol from sugar beets" Devel. Ind. Microbiol. 22: 719 (1981).

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INTRODUCTION OF METHODS FOR LARGE-SCALE PRODUCTION OF VACCINES IN DEVELOPING COUNTRIES

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## ABSTRACT

The large-scale production of vaccines is in several respects of another character than most other branches of biotechnology, that can be applied in the developing world. One of the reasons why this is so, is that pathogenic micro-organisms are used (although mostly not requiring a high class of containment), that are in many cases fastidious in their environmental conditions. Furthermore a very severe in-process and end-product control is required to ensure not only the efficacy (potency) but also the innocuity of the product, especially when not-purified products like wholecell suspensions are used. Finally, in contrast with most other biotechnological processes, the scale of production is always small, usually in the order of 100 - 500 litres, exceptionally increased to 1000 - 2000 litres.

Serious efforts have been made to increase the coverage of the world population with vaccines. The target of the EPI (Expanded Programme on Immunization) of WHO to immunize against six major infectious diseases has achieved hitherto not more than 20% world coverage.

Equally serious efforts have been made to introduce modern largescale methods of vaccine production in some selected Third-World countries. The selection criteria will be discussed. Adaptation of the western technology to the conditions in the Third World is only marginally possible, owing to some characteristics of this specialized branch of biotechnology. The reasons will be discussed.

In practice there is only a limited number of developing countries, with at least 50 million inhabitants and in which all other conditions are favourable, that are in a position to be promoted to a country fulfilling their need for vaccines by local production.

#### INTRODUCTION

The large-scale production of vaccines differs in several respects from most other branches of biotechnology, that can be applied in the developing world. Firstly, pathogenic micro-organisms are used (although mostly not requiring a high class of containment), that are in many cases fastidious in their environmental conditions. Secondly, a stringent in-process and end-product control is required to ensure not only the efficacy (potency) but also the innocuity of the product, especially when not-purified products like wholecell suspensions are used. Finally, in contrast with most other biotechnological processes, the scale of production is small, usually in the order of 100 - 500 litres, and never over 2000 litres.

### VACCINES

A vaccine is a preparation for active immunization against an infectious disease; it contains the antigen - or antigens - eliciting the desired immune response in man or in animal. Initially, the traditional vaccines - smallpox, rabies, later bacterial vaccines like diphtheria, tetanus, cholera, typhoid and pertussis vaccines - were produced with laboratory methods that cannot be classified under biotechnology, not even "avant la lettre". Development of modern methods of vaccine production, with the obvious introduction of cultivation in bioreactors - firstly for bacteria, lateron also for tissue cells as a substrate for viruses - followed the developments in the antibiotics industry. It became a "unit process" in its own right with several features distinguishing it from other branches of biotechnology.

The tables 1 to 4 list the main vaccines presently used. Table 5 shows the culture volume required to produce one total human dose. From these data an estimate can be made of the culture volumes needed for a certain quantity of final vaccine. It is striking that, in general, for live vaccines less culture volume is required. For other (medical) reasons an inactivated vaccine, provided it is efficacious, is often preferred. It is especially with regard to these vaccines requiring a relatively large culture volume, that large - scale cultivation is unavoidable with the introduction of proper bioreactors, control equipment and, in many cases, sophisticated downstream processing equipment.

## TABLE 1

# HUMAN BACTERIAL VACCINES

inactivated	live
Pertussis	BCG
Cholera	100000
Typhoid	Typhoid
Diphtheria	
Tetanus	· · · ·
*Meningococcal polysacch. *Pneumococcal polysacch.	
less important:	
Anthrax	Anthrax Brucella
E.coli	
Gas gangrene	
Leptospira	
Parapertussis	
*Paratyphoid	
Plague	Plague
Proteus	
Staphyloccci	
Streptoc.faecalis	

\*mixture of antigenically different species c.q. types

## TABLE 2

# HUMAN VIRAL VACCINES

inactivated	live
*Influenza	*Influenza
*Polio	*Polio
	Smallpox
	Measles
	Mumps
	Rubella
	Yellow fever
Rabies	The second state of the se
*Encephalitis	
Typhus(Rick.prowazeki)	Typhus

\*several antigenically different species c.q. types

# TABLE 3 VETERINARY BACTERIAL VACCINES

inactivated	live	
Tetanus	Anthrax	
* Clostridia		
Bruc.abortus		
*E.coli	Bruc.abortus	
Salmon.gallinarum		
Str.equi		
*Ps.aeruginosa		
Bordet.bronchiseptica		
Haem.pleuropneum.	al tentral	
*Campylobacter fetus		
*Leptospira		
Fusiformis necroph.		
Erysipelas		

\*several antigenically different species c.q. types

TABLE 4

VETERINARY VIRAL VACCINES

inactivated	live		
*Foot & mouth disease			
Newcastle	Newcastle		
	Marek		
Infect.bronchitis (IB)	1200 0. 0	100 m	
	Canine distem	per	
	Chicken pox		
	Pigeon pox		
	Rinderpest		
Hog cholera	the feature		
	and the second second		

\*several antigenically different types

# TABLE 5 CULTURE VOLUME FOR ONE TOTAL HUMAN DOSE BIOREACTOR CULTURE

vaccine	nr. of doses for 1 T.H.D.	ml cult.vol. for 1 T.H.D.
Pertussis	3	1
Diphtheria	3	• 0,3
Tetanus	3	0,3
Staphyloccocal toxoid	2	0,5
Cholera, parenteral	2	0,2
Cholera, oral	2	2
Typhoid, parenteral	2	0,02
Typhoid, oral	2	2
BCG	1	0,02
Meningococcal polysacch	. 1	2
Polio, inactivated	3	6
Polio, live	3	0,1
Smallpox	1	0,03
Rabies, inactivated	4	40
Measles, inactivated	3	6
Measles, live	1	1
Rubella, live	1	1

### EXPANDED PROGRAMME ON IMMUNIZATION

During the last fifteen years I have been involved in the transfer of know-how for the production of vaccines. It is obvious that for highly-developed countries the same or similar methodology and layout can be recommended as used in our Institute; for developing countries, however, the introduction of an adapted technology should at least be considered. I shall restrict myself in the following to human vaccines, of which I have most experience. A further restriction shall be that most of my considerations will be related to the six EPI-vaccines.

EPI stands for "Expanded Programme on Immunization". The idea to promote a worldwide campaign against six major infectious diseases came from WHO (1974). In 1977 a target for complete immunization of all children against tuberculosis, pertussis, diphtheria, tetanus, polio and measles in the year 1990 was set. Part of the policy is promotion of vaccine production, if advisable, on local (country) level. I think that of no other product of biotechnology a world strategy is so clearly established.

## WORLD VACCINE PRODUCTION AND IMMUNIZATION COVERAGE

I shall now try, with a few tables of recent data (partly obtained from WHO-EPI) to give you an impression how vaccine production is spread globally, and which stategy for the near future shall be applied.

Table 6 gives an impression of the use of the six vaccines in the six WHO-regions; please note that:

- immunization activities do not imply complete or even extensive vaccination
- many countries use imported vaccines

## TABLE 6

IMMUNIZATION ACTIVITIES BY WHO-REGIONS AS OF OCTOBER 1981 (FROM EPI-WHO)

Region	AFR	10	AM	RO	SEA	RO	EUR	10	EM	20	WPI	RO
nr. of countries	46		4	7	11	ins Un	37	-	24	1	3	2
teleconate era ma	nr.	%	nr.	%	nr.	%	nr.	%	nr.	%	nr.	%
Vaccines deliv.		11	mag	84 E.	30	1.13	anon.	2.0		100	15-10-2	14 3
- BCG	30	65	27	57	10	91	10103	aune	24	100	29	91
- DPT	30	65	47	100	10	91	bees.		24	100	32	100
- Measles	29	63	25	53	6	55	1	a .	24	100	17	53
- Polio	29	63	47	100	9	82	1.10	1.4	24	100	32	100
- T for women	26	57	20	43	9	82	a n	jus .	15	62	11	34

Graph 7 showing the incidence of three major diseases, leads to the conclusion that increase of vaccine application is badly needed. Table 8 gives an impression of the immunization coverage in the world. Graph 9 clearly indicates that a considerable percentage of the vaccines used is possibly not of dependable quality. GRAPH 7

Reported world incidence rates per 100 000 population for measles, tetanus and poliomyelitis, 1974-1980. (from WHO)



## TABLE 8

ESTIMATED PERCENTAGE OF CHILDREN IMMUNIZED IN THE FIRST YEAR OF LIFE AND PERCEN-TAGE OF PREGNANT WOMEN IMMUNIZED AGAINST TETANUS, BY WHO REGION, DURING THE LATEST PERIOD OF 12 MONTHS FOR WHICH INFORMATION IS AVAILABLE (1978-1980)(DATA FROM WHO)

Region	Percentage of popula- tion covered by reports ¥	Per	centage of by 12 mo	children im hths of age	munized	Percentage of pregnant women im- munized
	- retrant	BCG	DPT III	Polio III	Measles	Tetanus II
Africa	**				1	6
America	60%	54%	37%	34%	37%	10%
South-East Asia	19%	17%	15%	2%	0.1%	13%
Europe	**		100			
Eastern Mediter- ranean	99%	25%	22%	24%	31%	1%
Western Pacific	**			1		Deel -

\* Where percentage differs for different vaccines, the highest percentage is shown.

\*\* In these regions the information system to document these data is still under development.

## GRAPH 9

Quality of EPI vaccines being used; percentage of countries/areas by who regions



From the foregoing I have tried to estimate the coverage, for these six vaccines, in the world as a whole. Table 10 indicates that a dramatic increase in production of vaccines is necessary. It is, in this respect, striking that the author in an address given in Stockholm in 1964 when the world population was at  $3 \times 10^9$ , the vaccine coverage was estimated at 10 to 20%, a similar figure as the one I present you today! We seem hardly able to fill the gap caused by the world population increase!

### TABLE 10

## WORLD VACCINE COVERAGE FOR "EPI TARGET DISEASES"

Rough estimate present coverage:	: BCG 25%
	DPT 3x 20%
	Polio 3x 10%
	Measles 10%
	T (women) 5%
World population :	$4600 \times 10^6$
Birth rate :	: 2,9%
Births per year	$: 133 \times 10^{6}$
e.g. DPT needed :	: 400 x 10 <sup>6</sup> doses
incl. loss	: 550 x 10 <sup>6</sup> doses

In this context three expressions may be mentioned:

- vaccine need: the amount of vaccine needed for 100% world coverage.
- vaccine demand: the amount of vaccine the world is able to buy, which can be transported (cold-chain!) to the periphery, and be applied by capable people, mostly working in a national immunization programme.
- vaccine production capacity: the total installed capacity, which may exceed the demand because of shortage of money and lack of logistics of vaccine application.

### ROLE OF DEVELOPING COUNTRIES

How do the developing countries come into the picture for the large-scale production of vaccines?

First of all, long ago (beginning of this century) there were Institutes, frequently of colonial origin (e.g. Instituts Pasteur) having mostly a reasonable quality of medical bacteriological work. These institutes prepared vaccines such as smallpox and rabies, lateron bacterial vaccines by laboratory-scale methods. Large-scale production, to meet the demand for complete vaccination of the country, is virtually impossible with the old methodology. A million doses per year is the highest capacity I have ever encountered in such an Institute or laboratory.

## TABLE 11

		inh. 10 <sup>6</sup>	BCG	DPT	Т	м	Po1.
EURO	Austria	8		Х	х		X
	*Belgium	10	X		Х	X	X
	Bulgaria	9		Х	х		
	Czechoslov.	16		Х	Х	Х	
	Denmark	5	X		X		X
	Finland	5		X	X		
	*France	55	X	X	X	X	X
	EGermany	17	X	X	X	X	
	*WGermany	60	X	X	X	X	X
	Greece	10	X	X	X		
	Hungary	10	X	X	X		
	*Italy	60	X	X	X	X	X
	<b>Netherlands</b>	15	X	X	X	X	X
	Norway	4		X	X		
	Poland	40	X	X	X		
	Romania	20	X	X	X	X	X
	Sweden	8			X	X	X
	*Switzerland	6	X	X	X	X	X
	USSR	270	X	X	X	X	X
	*UK	60	X	X	X	Х	X
	*Yugoslavia	20		X	Х	Х	X

LOCAL PRODUCTION OF VACCINES (FROM EPI-WHO)

\* = exportation

## TABLE 12

starogram and the	inh. 10 <sup>6</sup>	BCG	DPT	⊂ <b>T</b> ⊃	м	P
AMRO Argentina	30	х	252.00	х	120	1
Brazil	130	Х	x	Х		101
*Canada	25	Х	X	х	X	X
Chili	11	Х	X	Х	11.20	
Colombia	25	x	X	Х		
Cuba	10	Х	x	х	LIST CAL	
Ecuador	9	X	x	х	20.11	
Guatemala	8		X			
Mexico	70	Х	X	Х	X	X
Peru	20	X	0.1.53		1.00	
*U.S.A.	230		X	Х	X	X
Uruguay	3	Х	X		10	
Venezue1a	20	х	X	X	-	là.

# LOCAL PRODUCTION OF VACCINES (FROM EPI-WHO)

# \* = exportation

Tables 11-13 give an impression of the local production of vaccines. Quantitatively, however, the data given are difficult to interpret because a country may claim the production of a given vaccine, while in practice it produces a small quantity only, sometimes not meeting WHO requirements or even local standards, provided they exist. It is striking that, not only in the developed world, small countries seem to have their own vaccine production. We have come to the conclusion that a developing country should have a certain minimal population to justify the local production of vaccines in such a quantity and of such a quality that complete immunization of the population is assured. We use, for that minimal population size, a figure of  $50 \times 10^6$  whereas WHO tentatively works with  $30 \times 10^6$ . For a population of  $50 \times 10^6$  inhabitants and a birth rate of 4%, the yearly need for e.g. DPT-vaccine can be calculated as follows:  $50 \times 10^6 \times 0.04 = 2 \times 10^6$  births per year

3 doses and 50% loss -> c.10x10<sup>6</sup> doses per year

TABLE 13 LOCAL PRODUCTION OF VACCINES (FROM EPI-WHO)

		inh. 10 <sup>6</sup>	BCG	DPT	т	м	Po1.
WPRO*	Australia	15	x	х	x		
	China	1000	X	х	х	X	x
	Japan	120	X	Х	X	X	X
	Philippines	50	X		X		15
	S.Korea	40	1.1	Х	X	X	1.10
	Vietnam	60	x	х	x		x
SEARO	Burma	40			x	1	1.0
	India	710	X	X	X		0
	Indonesia	150	X	x	x		0
EMRO	Egypt	45	X	x	х	1.1	0
	Iran	40	X	X	X	X	X
	Jordan	4		-	X		
	Tunisia	7	X			25.0	
AFRO	Madagascar	10	x			1 Star	2
	Senega1	6	X			1.4	-
	SAfrica	30	X	x	Х	X	X

0 = from imported bulk

\* = exportation.

This amount of vaccine can be produced in a plant of the size as present in the RIV, and which is shown on Figures 1 and 2. From Table 14 it can be deduced that, especially in African and Middle Eastern regions many countries do not yet participate in vaccine production.

In Tables 15-17 data, deduced from the foregoing tables on local production of vaccines, are used to indicate countries, of a given minimal population size, in which the local production of vaccine could be stimulated, provided a number of other conditions are fulfilled.



Fig. 1. Bioreactor room for bacterial vaccines at RIV. Shown are two bioreactors of 140 and 350 L cultivation volume, with control panels. Note the two pipelines - in the upper middle part of the photograph - making a direct connection between the culture vessels and the continuous centrifuge (see Fig. 2).



Fig. 2. Sterilizable continuous centrifuge. Connected directly (see pipelines in upper left corner) with the bioreactors, shown on Fig. 1. Capacity 200-300 L.h<sup>-1</sup>.

## TABLE 14 VACCINE PRODUCTION ACTIVITY PER REGION

Deduced from country tables for BCG, DPT, Tetanus, Measles and Polio

Region	Total Inhabitants	Inh. Vacc. Prod.Country	%
WPRO	1350	1285	95
SEARO	1075	900	84
EMRO	270	96	35
EURO	890	708	80
AMRO	640	591	92
AFRO	360	46	13

## TABLE 15

COUNTRIES NOT INVOLVED IN THE PRODUCTION OF ANY OF THE SIX "EPI-VACCINES", BUT HAVING A POPULA-TION OF MORE THAN 30 C.Q. 50 × 10<sup>6</sup>

> 50 x	10 <sup>6</sup>	30-50	x 10 <sup>6</sup>
country	inh. 10 <sup>6</sup>	country	inh. 10 <sup>6</sup>
Nigeria	82	Ethiopia	30
Bangladesh	94	Zaire	30
Pakistan	93	Turkey	48
Thailand	50	Spain	38

# EVALUATION

Let us focus now on the production of vaccines in the developing countries, and try to give an answer on the following questions: - what other conditions than country size have to be fulfilled

for successful introduction of large-scale production of vaccines?

- what contribution can developing countries make to the development

of this large-scale production.

- should the western technology be straightforwardly applied, or is adapted technology indicated?
- what successes have been booked and what failures have been made?

### TABLE 16

COUNTRIES NOT INVOLVED IN THE PRODUCTION OF DPT-VACCINE, BUT HAVING A POPULATION OF MORE THAN 30 C.O.  $50 \times 10^6$ 

> 50 x	10 <sup>6</sup>	30-50	x 10 <sup>6</sup>
country	inh. 10 <sup>6</sup>	country	inh. 10 <sup>6</sup>
Nigeria	82	Ethiopia	30
Bangladesh	94	Zaire	30
Pakistan	93	Turkey	48
Thailand	50	Spain	38
Philippines	50	Burma	40
		Argentina	30

Starting with the one but not last question brings us in the heart of the discussions of this symposium. For several reasons it is hard, in vaccine production, to adapt the technology to the conditions prevailing in a developing country. There is considerable disagreement among advisers over this point, leading sometimes to the oversimplified question: "should bioreactors be used or not?". It is a fact that ten million doses of bacterial or inactivated viral vaccine cannot be prepared without the help of a bioreactor. The alternative of cultivation in small units could be met with the cheap labour available in a developing country but often is bound to fail because of the high rate of contamination, the lack of control of the cultivation and consequently the impossibility to ensure a repeated high quality of the end product.

Another alternative proposed, namely to use "simpler bioreactors" is a fallacy, because especially in developing countries with, in many cases, a lack in mechanical and electrotechnical assistance,
a completely foolproof installation - with redundancies as in a modern aeroplane - is required, and that is neither a simple nor a cheap solution.

# TABLE 17

COUNTRIES NOT INVOLVED IN THE PRODUCTION OF POLIO-AND/OR MEASLES-VACCINE, BUT HAVING A POPULATION OF MORE THAN 30 C.Q. 50 x 10<sup>6</sup>

> 50 x 10 <sup>6</sup>		30-50 x 10 <sup>6</sup>		
country	inh. 10 <sup>6</sup>	country	inh. 10 <sup>6</sup>	
Nigeria	82	Ethiopia	30	
Bangladesh	94	Zaire	30	
Pakistan	93	Turkey	48	
Thailand	50	Spain	38	
Philippines	50	Burma	40	
		Argentina	30	
India	710	Egypt	45	
Indonesia	150	Poland	40	
Vietnam	60	Brazil	130	

Another point is the size of an installation, keeping in mind the slogan: "Small is beautiful", sometimes applied to applications of biotechnology in developing countries. As already mentioned, the size of cultivation, and hence of the subsequent downstream processing, for vaccine production is already relatively small. A further diminution in scale below 10, or say 5, million doses will increase overheads and the costs for in-process and end-product control to such an extent that it is becoming too expensive, even with low labour costs.

From our own experience in developing countries the following points have to be considered for the successful promotion of the local large-scale production of vaccines of good quality.

- a. The infrastructure of the country should be such, that there is a well-organized laboratory with experience in microbiological work. In exceptional cases the establishment of a new laboratory could be considered.
- b. The laboratory should have qualified staff, which is capable and willing to acquire - by fellowships abroad - not only the microbiological but also the technological know-how needed. A good criterium for the scientific standing of the staff is the presence of a good library (with subscriptions to major journals), that is frequented by the staff, and the existence of regular scientific meetings.
- c. Apart from the microbiological staff a good background of purely technical staff should be present. In practice this is in many cases the most difficult point. In most developing countries it is easier to find experienced bacteriologists than qualified mechanical and electrical engineers!
- d. Premises should be suitable, according to existing regulations, for the work with large quantities of pathogenic micro-organisms. Frequently, constructing a new building is cheaper than remodelling an old one. Supply of amenities like electricity and water should be well secured.
- e. The installation for the mass cultivation (bioreactors) and the further equipment for downstream processing and further steps (combining, filling) should be carefully chosen with special regard to the existing local conditions.

## DEVELOPMENT COOPERATION

In many instances a cooperation between a developing country and a donor country (bilateral) or international agency (multilateral) will take place for the realization of a vaccine production plant. Firstly, a feasibility study must be made, taking into account a.o. the points mentioned above. In the final plan the commitments of all parties concerned should be clearly defined and laid down in a contract, signed by all parties.

It is advisable to phase the activities, start with one vaccine only, and evaluate the success, in terms of quantity (production target) and quality (WHO-requirements) before commitment for another vaccine.

# TABLE 18

# APPROXIMATE TENDER PRICES OF HUMAN VACCINES, IN U.S. DOLLARS PER DOSE

DPT	\$ 0,03
Polio (Sabin)	\$ 0,03
т	\$ 0,02
BCG	\$ 0,01
Measles	\$ 0,12

Of course the financial consequences of the plan should be part of the feasibility studies. It is not absolutely necessary that the total investment of donor country (equipment, installation, manpower) and developing country (building, manpower) plus the operational costs once the plant is on full capacity should completely outweight the costs of buying the vaccine abroad (see Table 18), but a serious imbalance between the two should be considered as not conducive to final success for both parties concerned.

# CONCLUSIONS; NEW TRENDS

In contrast with some other biotechnological processes, adaptation of the western technology, in order to suit the prevailing condition in developing countries, is only marginally possible, mainly because a high-standard product is essential not only for efficacy, but also for safety or innocuity of the injected product. Furthermore the scale of production is already relatively small, so that there is a limit set, below which it is hardly worthwhile to erect a complete laboratory for vaccine production, because all the other prerequisites, as highly qualified personnel, good technical infrastructure, and the effort needed to control the final product remain virtually the same at smaller scale.

So only a limited number of developing countries, with at least  $50 \times 10^6$  inhabitants, and fulfilling the other conditions mentioned in the foregoing chapters, are in a position to be developed to a country, producing each year all the vaccine needed.

With these countries the western world could develop a close collaboration, not only for the benefit of these developing countries. The production of vaccines in such countries could well go beyond the present classical vaccines. It is well-known that, in several cases, genetic manipulation can lead to a methodology in which a simple micro-organism can produce a vaccine, that otherwise would need a much more complicated technique of cultivation. These methods are within the reach of a vaccine producing developing country, meeting the criteria mentioned earlier. Of course there are several extra conditions such as availability of the recombined strain, and possibly the special safety conditions required. The same reasoning does not apply if vaccines would be produced by complete synthesis, because this most probably requires another highly sophisticated set-up; moreover preparing vaccine by synthesis would no longer be biotechnology.

I am convinced that our own country - as some of the other western countries - is in a good position to properly stimulate the vaccine production in selected developing countries, and to develop a true collaboration, with benefits for both parties. Although not dealt with in detail in this paper, the dutch know-how on veterinary vaccines can equally play a role in this field.

I should like to finish with a thought developed recently by the present Minister of Development Cooperation of the Netherlands (the quotation is not litteral): "a dramatic change in the economic situation in our country, together with a dramatic change in behaviour of the Third World leads to a situation where developing countries more and more decide for themselves what is good for them. In such a situation development cooperation is a business of equal partners, and aid far below market conditions should be gradually avoided". When these countries the teators would could develop a class of incorrection not only for the sevelit of their developing on these functions of vacoling in such communications and beyond and predection of vacoling in such communications in reversit orange amounts termination and for the function the developing which a sumple allow underland or statistic s variations the distribuwould once a mount contribution and the second or the developing and all once a mount of an transfer in the second or the mount of the statistic termination and the second of the reacting and the statistic and the second of the second termination of the second of a second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second the second of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second termination of the second of the second of the second of the second termination of the second of the second of the second of the second termination of the second termination of the second of

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## ABSTRACT

The clonal propagation of oil palm is an example of successful transfer of biotechnology developed in the West and transferred to developing countries. The paper emphasises the need for true international collaboration combining the expertise and resources of the originators of the technology with the practical experience and local knowledge of the users. Long term success demands continuing technical support and collaboration to ensure that the new crops and processes are geared to the needs of the world market.

The report is illustrated with an account of the development of oil palm clones by tissue culture and some early results from Malaysian field trials.

#### INTRODUCTION

There are various contributions that technologically advanced Western nations can make to the developing economies of third world countries. Among the most effective and useful is the establishment of real business partnerships of mutual benefit to both donor and recipient country. Capital investment and technological expertise from the West, coupled with local knowledge and experience in the developing country can result in projects of economic benefit to both.

The Unilever/Harrisons & Crosfield\* development of new high performance clones of oil palm is a good example of such a partnership.

\* Unilever is a multinational holding company active in over 75 countries, with head offices in London and Rotterdam. Harrisons & Crosfield is an international company based in U.K., with major interests in plantation crops.

The purpose of this paper is to describe the development of the oil palm programme, and to show that success required carefully executed transfer of technology, and its continued reinforcement.

Although details of the propagation method were best worked out in UK with the technological resources of modern analytical methods, electron microscopy, DNA densitometry, NMR and so on, the technique is useless without access to a strong oil palm breeding programme, good agronomic trials, and the ability to identify palms of premium quality. Finally an efficient propagation unit, nursery and advisory back-up system is required in the recipient country. All these functions are best carried out locally by people with experience of the crop, and cannot be done remotely.

# USE OF TISSUE CULTURE OF CLONAL PROPAGATION

As the techniques of plant tissue culture developed during the 1960's the potential for rapid multiplication of plant varieties became apparent. Of course many valuable horticultural and crop species are already propagated vegetatively by conventional techniques using cuttings, offsets or grafts. In such cases the tissue culture techniques must compete in price or quality with what is already often an efficient process. In the case of oil palm there is no alternative clonal propagation method, and the industry relies on seed production.

Plant tissue culture provides an opportunity for palm breeders to select unique individuals from their genetic recombinants and to perpetuate them as clones of vegetatively propagated plants. Each clone will faithfully reproduce the genetic characteristics of the ortet\* from which it was derived. In this way it will become possible to establish distinct varieties of oil palm analogous with the range of varieties available in other vegetatively propagated species such as apples.

\* The ortet is the source plant from which a clone is derived, the individual members of a clone are called ramets, and different clonal lines are known as genets.

	Toni seed	nes/ha 1	% Oil	T/ha Oil	
Soya	2.5		20	0.5	
Groundnut	4.5	(in shell)	20	0.9	
Cottonseed	2.5		20	0.5	
Sunflower	2.0		40	0.8	
Rapeseed	2.5		40	1.0	
Sesame	1.0		50	0.5	
Coconut	4.0	(copra)	65	2.6	New hybrids
Oil palm	23.0	(bunch)	24	5.6	Best Malaysian

TABLE 1 Best Average Yields of Oilseeds

From: Corley (ref. 1)



Fig. 1.

 Distribution of oil yields of individual palms in a D x P progeny trial (mean of 2 year's data).

The advantages of uniform clonal material are already exploited in the rubber industry, where bud grafting is used, and of course in crops such as potatoes, soft and top fruit, where planting seedlings is an activity confined to plant breeders seeking new variation.

#### Potential for oil palm improvement

Great improvements in yield and quality of palm oil have been achieved by conventional palm breeding programmes, to make oil palm the world's most productive oil crop. Good Malaysian plantations can now produce annual average yields of 6 tonnes of oil per hectare, compared with 1 tonne of oil ha<sup>-1</sup>.yr<sup>-1</sup> or less for many annual oil crops (Table 1).

Nevertheless, because the oil palm is a naturally outbreeding species breeding parents carry a high degree of heterozygosity (fortunately so, since most current breeding stock is from a very restricted ancestry). Consequently even good progenies from high yielding parents show coefficients of variation for yield of 20% or more. Fig. 1 shows data obtained by Corley (ref. 1) from a Dura x Pisifera progeny trial in Malaysia, averaged over a 2 year period. Such variation is typical of that obtained from wellconducted trials in which the environmental component of variability would be expected to be low. From data of this type it could be predicted that clones derived from among the best 5% of the tenera progenies should yield at least 30% more than the progeny mean.

On the basis of a 30% yield improvement it will be possible to sell clonal plants at an economic price which will, over a period of years, recoup the research and development costs. Oil palm planters, like farmers world wide are shrewd commercial people who are unlikely to pay premium prices for clonal plants unless they can obtain improved performance from their crop. The anticipated yield increase will ensure that any increased planting costs are recovered within the first couple of years of the crop coming into bearing. Thus assuming the success of the programme, Unilever can obtain a reasonable return on its investment, while the grower obtains a higher yield.

# Tissue culture

The majority of commercial tissue culture laboratories propagate by micropropagation, starting from shoot tips or lateral buds containing leaf primordia subtending vegetatitve bud-initials which can be stimulated by suitable application of cytokinins to proliferate exponentially. By this means, disruption of the organisation of the bud meristem is avoided, chimaeras are faithfully reproduced and the frequency of 'sports' is no higher than using conventional clonal propagation methods. Very large numbers of plants of many different species are produced each year using this system.

So far attempts to employ this method with palms have failed. Culture of the apex usually yields only a single shoot, and the leaf primordia close to the apex are many months and cell generations away from initiating axillary buds, which in any case are destined to become inflorescences. Nor has it proven possible to divert established flower primordia into vegetative buds.

All reports of success so far have involved the intermediate formation of a callus on the primary explant. Callus is the term given to the relatively disordered proliferation of cells resulting from stimulus with auxin type growth substances such as 2,4-D or NAA. Callus formation is a natural response to wounding in most dicotyledons, under the influence of locally produced natural auxin, IAA. This mechanism appears to be absent or very weak in monocots, and callus induction is considerably more difficult, often requiring between 10 and 100 fold higher levels of growth substances. Any actively growing tissues can be used to initiate callus, and successful cultures have been established from roots, young inflorescence and leaf base tissues. The initial callus formation in oil palm is usually in the form of partially disorganised root primordia: nodular in 2,4-D or recognisably root like in NAA (ref. 3). After repeated subculture some cultures develop a faster growing friable callus consisting of small, active meristematic centres which shed off cells that rapidly enlarge, vacuolate and cease division, eventually becoming senescent with shrunken contents, and ultimately bearing empty cell walls.

Occasionally some cells within these calluses undergo a further transition and multiply to form embryo-like structures termed embryoids. Under the right cultural conditions they will germinate and grow into balanced plantlets with root shoot, haustorium (cotyledon) and all the features of zygotic embryos. More frequently, and more usefully, however they form proliferating masses of hard nodular tissue which gives rise to numerous shoot primordia. Paranjothy (ref. 5) has compared this tissue with orchid protocorms, which indeed it resembles.

This material is used as the starting point for the multiplication cycle in the production of oil palm clones, following the scheme described by Lioret (ref. 4) (Fig. 2). The sequence of callus induction through to embryogenesis is at present slow and unpredicatable. Fortunately, once it has occurred it is possible to multiply unlimited numbers of plants by a method similar in practice (although different in principle) to micropropagation. Shoots are removed from the proliferating cultures and placed in rooting medium, subsequently to be hardened off and planted out. Meanwhile the remainder of the culture is recycled to continue proliferation. Growth regulators must be carefully balanced to maintain this process, and optimum levels are not only different for each genotype, but change with time as cell lines become "habituated".

In the majority of species studied, callus formation results in the formation of cytologically abnormal cells, and frequently plants regenerated from calluses show a wide range of variation. This appears particularly prevalent in polyploid species such as Figure 2

Propagation of oil palm by tissue culture



wheat, potato, tobacco, and less frequent in diploid species such as barley. Oil palm is no exception in showing cytological instability at the callus stage, but the embryoids so far examined appear uniformly diploid, and the clones derived from cultures show a high degree of within-clone uniformity.

#### TECHNOLOGY TRANSFER

In the early stages of our programme it was easiest to start from aseptically germinated seedlings and excised embryos. Once a successful technique was established it was of course essential to sample from selected ortets with the required genetic constitution. The first experimental clones became available for field testing in early 1975 and about 40 different genotypes were produced from seedlings derived almost entirely from D x P hybrid seed produced on our Cameroon plantation.

Dr. R.H.V. Corley, a palm physiologist with long experience of the crop, set up the first Clonal Oil Palm Research Unit in Malaysia at the beginning of 1976 with a consignment of clonal palms from Colworth House. A first priority was to check that the clonal palms behaved normally in every way and gave rise to normal palms. Equally important, we needed to establish that clones are significantly more uniform than seedling populations. The first trials with clonal palms soon dispelled doubts on these counts (ref. 2) but since the early clones were from seedling derived cultures there was no expectation of improved yield.

We now have data on the yield up to 42 months from planting, for 11 clones. Tables 2 and 3 show that individual clones are highly distinctive in their yield and bunch characteristics. High yield at this early stage is more an indication of early fruiting than a guarantee of long term high productivity, but nevertheless high early yield generates valuable early cash flow. Other distinctive clonal characters are seen in the ratios of kernel and mesocarp oil in the fruit, reflecting the relative sizes of these components (Table 3). Carotene levels (Table 4) also show characteristic differences in these clones, falling fairly clearly into high (about 1200 ppm) and low (about 600 ppm) groups. Carotene rich oils would be favoured for local consumption in Africa, whereas oil for refining should be as pale as possible to minimise the bleaching requirements. The variation seen between these unselected clones reflects the range of variation normally present in seedling populations but not noticed because oils from different trees are normally mixed in the extraction process. Fruits from suitable sized plantings of single clones could be extracted separately and kept as distinct products to provide oils of different quality for different processes.

# Ortet\* Selection

Although good clones may appear by chance from unselected lines, it is far more effective to select the best ortets, so that clones are produced from palms known to have the right combination of characters for high yield combined with, for example, disease resistance, oil quality, drought tolerance and so on.

Although selection of suitable ortets for cloning might appear straightforward, it is not simply a matter of sampling from the "best" palm in a plantation. There are few individual palm records in commercial plantings, and high yielding individuals are frequently growing in favoured sites, possibly vegetatively vigorous and thereby dominating their neighbours. Suitably recorded palms are only available in progeny trials in well established palm breeding programmes. Of course the currently most advanced germ plasm is found amongst these progenies. Clones derived from such material will represent the best breeding lines available.

\* See footnote on page 142.

TABLE 2

Clone	No. of palms	Oil	Kernel	Oil + Kernel
932	3	15.93	7.12	23.05
997	34	13.76	1.96	15.72
926	54	13.23	2.01	15.24
924	28	10.25	2.41	12.66
Seedling control	90	9.32	4.49	13.81
905	3	8.98	3.65	12.63
976	2	7.21	5.18	12.39
960	4	6.85	2.77	9.62
931	60	6.40	2.18	8.58
975	31	5.95	2.66	8.61
970	3	5.13	2.47	7.60
949	30	4.90	1.60	6.50
907	5 5	4.75	0.39	5.14
939	5	1.35	2.78	4.11

Oil yields from clonal palms (kg/palm) up to 42 months from planting.\*

\* Results from 4 separate plantings. Clone 926 was included in all 4; yields of other clones were expressed as a percentage of that of Clone 926 in each planting, weighted mean percentages were calculated, and the overall yield for each clone estimated from this percentage and the mean yield of Clone 926. The correlation between the yields of five clones in two different plantings was r = 0.968 (p 0.05, 3 d.f.)

#### TABLE 3

Bunch characters of five oil palm clones

values 905	of fruit 926	characters 932	for va 939	arious clones 997
1		Harden and State State State		PERSONAL ALL A
56.7	46.1	78.2	47.0	48.4
55.5	56.0	71.7	48.2	51.3
79.4	88.6	74.7	54.8	90.8
11.6	4.7	9.4	19.1	4.7
5.0	5.5	13.4	14.8	3.7
	56.7 55.5 79.4 11.6 5.0	56.7   46.1     55.5   56.0     79.4   88.6     11.6   4.7     5.0   5.5	56.7   46.1   78.2     55.5   56.0   71.7     79.4   88.6   74.7     11.6   4.7   9.4     5.0   5.5   13.4	56.7 46.1 78.2 47.0   55.5 56.0 71.7 48.2   79.4 88.6 74.7 54.8   11.6 4.7 9.4 19.1   5.0 5.5 13.4 14.8

Data from palms at 42 months after field planting.

TABLE 4

Clone		Carotene content ppm	
905		1344	
907		357	
924		1056	
926		640	
931		1158	
932		1289	
939		644	
949		1590	
970		634	
975		1093	
976		583	
997		1204	
	S.E.	188	

Carotene content of oil from different clones

As the technique of clonal propagation becomes available the emphasis of palm breeding programmes is changing from a need to produce uniform seed progenies to increasing the range of variation from which to select ortets for cloning. The future development of improved clones must go hand-in-hand with breeding programmes aimed at combining together the best features available from world wide genetic collections. These programmes require international collaboration in making germ plasm available and in setting up comparative trials in different parts of the world. The availability of clones allows us for the first time to examine the extent of genotype environment interactions in detail, by planting identical genetic stock in a range of different environments.

#### Future Clonal development

The international scope of the Unilever - Harrisons & Crosfield partnership allows us a unique opportunity to conduct world wide clone trials to uniform design that can be statistically analysed to yield results that can be interpreted with confidence. This development of international trials and exchange of germplasm is another contribution that can be made efficiently by industrial enterprise from the technologically developed countries. Although international agencies and foundations exist for improvement of many major crops such as cocoa, cassava, rice, there is as yet no such agency for oil palm improvement. Provided the individual nations are willing to collaborate with industry, rapid progress can be made to the mutual benefit of all. The initial requirement is for access to germ plasm and the establishment of comparable trials. Naturally, appropriate agreements must be negotiated to safeguard individual breeders or national rights to genotypes selected for commercial clone production and adequate quarantine safeguards must be assured. Although plant breeders rights are well established for most important crops in Europe and USA, no such schemes are yet internationally agreed for the worlds major tropical crops.

Export of palm oil provides a major source of foreign exchange for many developing nations. Traditionally, crude palm oil was exported direct to the industriallised user nations, where it was fractionated refined and blended for a wide variety of different applications. The major quality requirements at the point of export were limited to low free fatty acids and low oxidation levels, which could be achieved by good harvesting and mill management.

Increasingly the producing nations seek to undertake their own fractionation and refining, and to sell processed oils to the manufacturing countries. Unless there is close technical collaboration between producers and users there is a danger that inappropriate methods will be used to produce oils of inferior quality that cease to compete with alternative sources. The major users of palm oil can contribute directly by maintaining a close dialogue with the producer nations to ensure that the most up to date extraction and refining methods are introduced and that the products are tailored to the market, both present and future. Again such developments can best be achieved if developing countries remain open to capital investment by international companies directly involved in oils and fats processing. They are the major customers buying vegetable oils on the world markets, and they know the types and qualities of oils required in their manufacturing processes.

The development of clonal oil palms gives us an added opportunity to influence the markets available to palm oil. Increasing quantities of palm oil are being produced, and will soon saturate the demand for solid vegetable fats. The large market for liquid oils is only accessible to the more unsaturated fractions of palm oil. Hence development of more liquid oils would increase the market available to palm oil.

More liquid oils are available in the related South American oil palm species, <u>Elaeis oleifera</u>. Unfortunately although bunch yield can be quite high, low oil to bunch ratios render the use of <u>E. oleifera</u> uneconomic. There is sufficient inter species fertility to allow hybridisation, and there are now many well established trials of <u>E. oleifera</u> x <u>E. guineensis</u> hybrids, some of which have promising yields. The oil composition of the hybrids is intermediate between the parental types. Of more potential interest are backcrosses between the F<sub>1</sub> hybrids and <u>E. guineensis</u>, where widespread genetic segregation occurs and some individuals combine the yield features of <u>E. guineensis</u> with the quality characters of <u>E. oleifera</u>. These unique genetic combinations are ephemeral unless the individual genotype can be perpetuated by vegetative propagation.

Tissue culture can provide the means of production of totally new types of oil palms producing oils quite different in quality from traditional palm oil.

# Scale-up of propagation

At the laboratory level the techniques of plant tissue culture appear relatively simple and can apparently be operated as a low technology extension of horticultural practice. In reality they embody a great deal of sophistication. Hygiene is of paramount importance. Contaminant microorganisms can quickly overgrow plant cell cultures, and perhaps more important, slow growing contaminants are easily overlooked and can quickly spread by subdivision of infected cultures and by contamination of instruments. Hence a high standard of microbiological cleanliness and monitoring is essential, and individual transfer operators must be constantly vigilant.

Many plant cell cultures are sensitive to chemical contaminants in their environment. Residual traces of detergents on glassware, or small quantities of heavy metals in water can be lethal, so can solvent vapours from paints or adhesives used in growth room construction. Thus all stages of medium and glassware preparation and the growth environment must be carefully supervised.

Tissue culture media are complex and to be successful need reliable sources of chemicals, accurate weighing and preferably some analytical quality control.

For a successful scale up of tissue culture propagation these principles must be extended beyond the laboratory situation, where awareness of these requirements is part of the professional scientists' expertise, to the factory level with heavy pressure on throughput and with relatively untrained operators.

Effective scale-up becomes a problem of achieving an efficient low cost, high volume throughput without sacrifice of quality and hygiene and maintaining effective quality control through whatever crisis develops.

We have set up 2 development units, one in Malaysia, and one in UK. to undertake the R & D work required to take the existing laboratory procedures up to a reliable production system. Initially it is sensible to centralise the sophisticated high technology aspects of the process in technologically developed areas where for example uninterrupted power supplies can be relied upon to maintain close control of temperature, run autoclaves at effective temperature and pressure, provide adequate supply of distilled water, maintain filter fans on laminar flow cabinets and so on. Supplies of chemicals and glassware are also easier to obtain than in many tropical countries. Once plantlets are produced that can be transferred to soil and handled like seedlings, of course there is no substitute for the tropical nursery in the growing region. At some stage therefore plantlets must be transferred from the central production unit to the growing region, and this is a costly and vulnerable stage in the cycle, where heavy losses can occur if the plants are mishandled or delayed.

As the programmes develop, there will be larger scale requirements for clonal palm plants in some individual countries, and there will be a need for production to be transferred from the central tissue culture laboratory to the area where the palms are to be grown. This is a stage requiring a great deal of careful technology transfer. Although expertise in laboratory tissue culture techniques can easily be acquired in numerous courses and technical workshops, the success or failure of a new production unit will depend not only on scientific and technical skills but on management, staff training and recruitment, on maintenance of services and supplies and on rigorous quality control. The countries where the technology is developed have a major role to play in training managers from the developing countries so that they are in a position of awareness to provide the right environment within which the technical needs can be met. There are many difficulties in setting up a technically advanced process in an undeveloped area. Well trained local managers, aware of the local situation may be in the best position to overcome the problems, but they will need continued strong technical support and advice from the centre.

Thus we see a dual role for the central R & D production units such as Unifield TC in Bedford.

They have the full responsibility for collection and initiation of cultures, the optimisation of media and conditions for regeneration of individual genotypes and the development of effective scale-up methods.

They also have to produce sufficient numbers of plants for large scale commercial trials and subsequent commercial production. By retaining a centralised research unit we can ensure that the best possible use is made of the available germ plasm. Trials in different environments can be directly compared, so that hitherto unresolvable problems such as the evaluation of environmental and genetic components of variability, and the extent of variety x genotype interactions can be answered. They will thus be able to advise with confidence on the best material for any region, and where necessary to supply clonal culture stocks for multiplication. They can also maintain an independent clone bank, and technical back-up service so that if any local production difficulties arise perhaps due to power failures, or epidemic infection, they can be quickly overcome and any lost clones replaced.

For those countries where large numbers of clonal plants are required, such as Malaysia or Indonesia it is clearly sensible to set up local production units to serve those individual markets. In other cases, where demand is less, and liable to fluctuation it is clearly impractical to set up a unit, that to be cost effective must produce in excess of a million plants per year at a steady rate. For countries with a need for small one-off orders of top quality clonal material it is sensible to purchase from a central production unit located in an independent country. It will be essential to provide, in the recipient country, local centres of expertise to undertake the delicate stages of hardening and establishment of the young clonal plants and to supply local growers with advice on fertiliser regimes and husbandry.

Unifield will therefore be ideally placed, close to the extensive research facilities at Colworth House, yet directly in touch, through its international clonal palm field trials, with potential users.

Colworths' present role is to further improve the existing technology, and to develop new approaches, for example in the use

of liquid culture techniques and the application of molecular genetics, so that continuing improvements in cost and quality can be made.

Also under development at Colworth House, are methods of cryopreservation of cultures so that clones in culture can be maintained indefinitely in a deep frozen state. Access to liquid nitrogen and reliable technical resources available in the developed nations are good reasons for maintaining a complete clone bank in the West.

Of course, as distinct varieties of oil palm are developed using the cloning process, it will be important to recognise and safeguard the rights of the plant breeder originating the ortet that gave rise to the clone.

Recognition of Plant Breeders Rights is now well established for most major temperate crops and many horticultural subjects. It will take some time to set up similar schemes for tropical crops, and will require a great deal of mutual trust and goodwill. As a first step it is essential that individual clones should be clearly identifiable. All the clones we have so far established possess a number of distinctive features in vegetative habit, bunch characters and oil composition. These diagnostic features can be further reinforced by biochemical fingerprinting using protein electrophoresis. It may eventually be possible to unequivocally identify the patterns of DNA present in each genotype. This will mean that individual clones can be easily recognised and can form the basis for palm breeders either to retain exclusive use of clones derived from their material, or to neqotiate a fair royalty on plants sold.

However, one of the great potential advantages of tissue culture is the ability to eliminate disease organisms thus safely to transfer germ plasm across quarantine boundaries. The coordination of international clone trials from an independent centre provides an unique opportunity for crop improvement, and it is to be hoped that individual countries will collaborate in allowing free exchange of germ plasm for their mutual benefit.

The long term success of the programme therefore depends on continued mutual development and collaboration. Palm breeders in the tropics will be encouraged to create novel recombinations of genes that can be stabilised in the form of clones. Centrally coordinated International trials can evaluate the new clones under the widest possible range of conditions to ensure the best adapted material is available for any situation. The trials themselves of course depend on good agronomists to ensure good trials management in each country. The central tissue culture unit will provide new tissue culture clones for trial and eventually for commercial multiplication and also an essential technical advice service and back-up clone bank. Without a reservoir of scientific and technical skills from which they can draw help and advice individual production units could quickly lose their whole production as a result of an unforeseen mishap. It is therefore essential to develop long term collaborative programmes to ensure that the combined resources of both producers and users of palm oil are used to mutual benefit in improving both yield and quality of the crop.

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# REFERENCES

- 1. Corley, R.H.V. (1981). Jour. Perak Planters Assoc. (1981): 35-49.
- Corley, R.H.V., Wong, C.Y., Wooi, K.C. and Jones, L.H. (1981) in "Oil Palm in Agriculture in the Eighties". Incorporated 2. Society of Planters, Kuala Lumpur, 1981. Jones, L.H. (1974). Oil Palm News <u>17</u>, 1-8. Lioret, C. (1981) in "Oil Palm in Agriculture in the
- 3.
- 4. Eighties". Incorporated Society of Planters, Kuala Lumpur, 1981.
- 5. Paranjothy, K. (1982). PORIM Occ. Paper. Palm Oil Res. Inst. Malaysia No. 5. A Review of Tissue Culture of Oil Palm and other plams.



Biotechnology in Developing Countries aims to be more than the cleverly chosen combination of two hot topics of today.

It is obvious that Biotechnology, as the technology of using microorganisms and tissue cells, has certain intrinsic advantages over other technologies in the setting of a Developing Country. In fact not only beer and wine making but also the preparation of fermented foods are classical examples.

With the tremendous needs of the Developing World of today there is of course more: not only the newer developments at the countryside like algal ponds and biogas installations, but also the greater sophistication of Rhizobium starters and cloned oil palms, for which well equipped laboratories are indispensable. It is questionable whether the classical fermentation technology, with bioreactors with capacities in the order of hundreds of litres for among others vaccine production, and of hundreds of kilolitres for antibiotics, can be adapted to the conditions prevailing in the Developing World. Six selected specialists from the Developing and from the Developed World give their vision on the prospects of Biotechnology, as a relatively soft technology, for the enhancement of the prosperity of the Third World.