MICROBIAL GENE TECHNOLOGY

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1. Biotechnology status in India present and future

P.K. GHOSH

Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services. It also covers utilization of microbes and microbial technlogy for the largescale production of useful products of food and pharmaceuticals. Biotechnology is as old as our civilization. However, modern biotechnology can be said to have taken off from early 1970s and has been accelerating exponentially ever since.

Modern biotechnology stands on the understanding of molecular basis of biological cell functions and the ability of mankind to alter the cell functions to make it produce products required by society. The technology is technique-based. The major new techniques available with modern biotechnology are recombinant DNA technology (r-DNA), hybridoma cell fusion, various sophisticated assay procedures like RIA, ELISA, etc., electropheresis, plant and cell culturing techniques and various intricate and sophisticated separation and purification methods of biomolecules such as precipitation by salts and solvents, high resolution chromatography, continuous solvent partitioning and gel filtration, etc. The new biotechnology holds potentials for developing products and processes in various sectors of agriculture, animal husbandry, aquatic life forms, health care, energy and environmental protection. Hybrid high yielding seeds, artifical seeds (stress tolerant and disease resistant), tissue cultured propagules of economically important plant materials in vegetables, fruit plants, orchids and ornamentals, commercial plants (tea, coffee, rubber), etc. would be available in years to come in the field of agriculture. In

fact, hybrid high yielding seeds and tissue cultured propagules of several economically important plant varieties are already available commercially. High milk or meat producing animals would be raised through foster mothers. Several transgenic aquatic life forms shall evolve to meet the growing protein requirement of mankind. In health care area, there would be significant developments in diagnosis and therapy of various historic incurable diseases like cancer, AIDS, infertility, diabetes, blood clots, heart attacks, blood pressure, healing of various wounds, etc. Solutions for combating several infectious diseases like leprosy, typhoid, cholera, diarrhoeal diseases, various water borne viral diseases, etc. would also be found. Already genetically engineered Hepatitis B vaccines, recombinant insulin, human growth hormone and recombinant interferon are commercially available. In the area of waste management, efficient anaerobic strains for better conversion of wastes into biogas shall be a reality.

An essential component for the assimilation, absorption and adoption of procured technology in the field of new biology is to have a strong R&D back up as the area is highly science-based. Entrepreneurial success may come about only if a strong R&D group is consistently stretching supportive efforts to any product or process commercialized. Industries are therefore either required to set up their own elaborate R&D units or create linkage with R&D institutes/ universities so that constant and steady flow of scientific inputs into the finished products or processes are ensured. As Indian industry has put comparatively less effort on R&D and as the business climate in this country is primarily seller-based, the innovative and highly sophisticated biotechnology industry has not yet been able to make a deep impression into the minds of Indian industrialists. Whatever infrastructure and awareness has been developed is through the efforts of the government, through its various programmes in several ministries and their institutes. Government funded institutes and universities in various corners of the country are engaged in R&D in all facets of new biotechnology, namely, cell culture, cell fusion, immunology, genetic engineering, fermentation technology, etc. and such R&D efforts have started showing results in certain sectors.

The promising results obtained in some sectors have not, however, been commercialized yet in any significant way. The main reason for this is the lack among Indian industrialists of sophisticated R&D infrastructure, knowledge base, and finally, the currently uncertain and unpredictable market conditions. However, this situation is gradually changing towards betterment and some industrialists—a few of whom are leading ones—are now showing interest.

As new biotechnology is only over a decade old, the products and processes commercially exploited currently world over are not many. Consequently, more often than not, such technologies are usually not available for transfer. In other words, to reap the benefits of new biotechnology, the country would need to develop its own technology. Technological priorities vary from nation to nation. In our country R&D efforts need to be strengthened and focussed in the following areas.

A. AGRICULTURAL AND PLANT BIOTECHNOLOGY

I: Hybrid High Yielding Seeds and Artificial Seeds

Conventional as well as biotechnological methods for the production of hybrid high yielding seeds would have to be used in industrial scale for crops and other agricultural, horticultural and forestry plants of economic value. Currently, the national average per hectare yield of most of the agricultural products including oilseeds, pulses and other food crops are low compared to standards in the developed world. Realizing this, several entrepreneurs have started importing hybrid high yielding seeds developed by conventional biotechnological methods for sale to cultivators with a view to increase the productivity as well as quality. Entrepreneurs are concentrating on bringing hybrid high yielding oilseeds, e.g. sunflower and agricultural crops like onion, potato, cabbage, etc. However, the basic technology for the development of hybrid high yielding seeds including development of male-sterile cell lines, etc. are yet to come to the country.

Development of artifical seeds consisting of tissues cultured embryos encapsulated in protective coatings is an emerging area for the production of unlimited individual propagules and maintenance of endangered genetic materials and other propagules. Work in this direction has already been taken up abroad. Such efforts need to be put in this country also.

II: Tissue Cultured Propagation

While techniques for tissue culture propagation of several plant materials have been perfected in this country, only two units have started producing on commercial scale some selected varities of agricultural plants and flowering plants. Tissue cultured plants of cardomom and banana are available commercially in this country. A few flowering plants are also produced by this technique and marketed. However, the techniques have vast scope in several of the plant species

and horticulture where technologies are being perfected in the country. Such technologies specially in horticulture have been perfected abroad and are available for sale. Entrepreneurs willing to procure such technologies from abroad are encouraged wherever these are beneficial to the country.

III: Invitro Production of Secondary Metabolites

The potential use of plant tissue culture for the production of secondary metabolites has been viewed with great optimism. Such metabolites may be pharmaceuticals, dyes, flavours, fragrances, pigments, etc. This area has high commercial application potential and needs much R&D efforts for developing commercial processes for continuous production of the secondary metabolites.

IV: Blue Green Algae (BGA) for Improvement of Soil Health

Certain BGA can grow and colonize on alkaline/saline soils and bring improvement in their physiochemical properties, viz. reducing in soild pH, electrical conductivity, exchangeable sodium and increase in solid aggregation while enriching soil with organic nitrogen. Certain nitrogen fixing BGA secrete some polysaccharides which increase water-retention capacity of soil and such BGA can be used as soil-conditioner for arid and semi-arid regions. R&D efforts in these areas need strengthening.

V: Germplasm Bank

A number of wild plant species and their natural habitat are disappearing as a result of urbanization and increasing area of the cultivated land. Thus, there is a general fear that the valuable germplasm is being lost irretrievably. As a result of recent advances, the cell, tissue and organ culture methods are considered as a means for long term storage of endangered germplasm through cryopreservation technique. Such techniques may be intensified in the country.

VI: Biofertilizers

Certain microorganisms like Rhizobium, Azotobacter, Azospiritum, etc. have the capability of fixing atmospheric nitrogen. Rhizobium inoculants can fix under optimal conditions 50 to 100 kg of nitrogen per hectare of land. Azotobacter inoculants serve the dual purpose of meeting the nitrogen requirements as well as protecting the crop from fungal diseases.

Leguminous plants including pulses, soyabean and groundnut are involved in symbiotic nitrogen fixation by Rhizobium bacteria. The natural varieties are not always of the required efficiency. Consequently, efficient bacteria are to be selected and maintained. The commercial packets of Rhizobium culture must be from the selected highly efficient packets of efficient Rhizobium cultures can be sold at quite a low prices and the requirements of the culture for seeds to be sown in one hectare land would work out to less than Rs. 10.00. The consequential increase in crop productivity is usually more than 20%. Benefits are thus substantial.

Azotobacter inoculants are recommended for cotton, wheat, mustard, etc. The nitrogen fixing capability is low (10-20 kg of nitrogen per hectare); however they help in other ways. Azospirilium inoculants can save considerable chemical nitrogen in crop like gowar, bajra, ragi, barley, etc.

Technology for the production of biofertilizers has been developed in the country to a level which needs further perfection on largescale. Several commercial plants set up could not always deliver quality material in the hands of farmers. The products are perishable with short shelf life and are susceptible to harder climatic conditions. These shortcomings need to be solved.

Stable products resisting harder climatic conditions with longer shelf life would be acceptable to Indian conditions. Technologies having such attributes alongwith microorganism possessing efficient nitrogen fixing capabilities are to be developed or procured.

VII: Crop Specific R&D

For catering to the needs of the growing population, the estimated requirement of food crops would be around 240 million tonnes by 2000 AD. Given the constraints of land and other inputs such as fertilizer, water, etc. such targets can only be achieved through modern techniques of genetic engineering and biotechnology. Recognizing the potential of the latest developments in biotechnology for improving agricultural productivity in a substantial manner, the Department of Biotechnology has identified the thrust areas for R&D in respect of selected crop plants. These are brassica, rice, wheat and chick pea. Specified goal oriented projects involving a collegium of institutions will be supported by the Department of Biotechnology under this programme in active collaboration with Indian Council for Agricultural Research. It is anticipated that these efforts would produce good results which would be visible with a decade.

VIII: Major Indian Institutes Engaged in Agricultural and Plant Biotechnology Research

The major institutes/laboratories engaged in programmes in agricultural and plant biology or biotechnology and the areas are given below.

The National Chemical Laboratory, Pune (NCL) has isolated virus-free sugarcane and banana clones and multiplied them through plant tissue culture techniques and the same are under large scale fields trial. It has micro-propagated cardamum, turmeric, tamarind, pomegranate, forest trees like teak, eucalyptus, various fruit trees and Salvadora. In some cases, the technology developed has already been transferred for commercial exploitation or are ready for transfer and some others are under advanced stage of field testing. NCL is also engaged in in-vitro propagation of high yielding coconut through tissue culture/somatic embryogenesis.

At the Indian Agricultural Research Institute (IARI), New Delhi, the Biotechnology Centre is engaged in gaining insight into the molecular mechanisms of such processes as are vital to finding application of molecular and biotechnological approaches to crop improvement objectives. The thrust areas include plant cell genetic manipulation, organization and expression of plant genome, plant microbe interaction and micro-propagation.

The National Botanical Research Institute is working on plant tissue culture propagation of various ornamental plants like bougainvillea, chrysenthemum, orchids and some medicinal plants like dioscoria, solanum, etc.

The Tata Energy Research Institute (TERI), New Delhi is running programmes in isolation of large plasmids from Rhizobium meliloti, molecular biology of nitrogen fixation in cyanobacteria and bacteria transposable elements of maize genetic transformation of tobacco, maize, etc. TERI is also working on tissue culture techniques for the propagation of medicinal plants.

At the Indian Institute of Technology, Kharagpur, a group is working on microbial delignification of agricultural residues, production of cellulase from locally screened fungus.

The Bose Institute, Calcutta, is using the tissue culture technology for generating plants from single cells (both haploid and diploid) protoplasts and fused hybrid protoplasts.

At the University of Delhi, the Department of Botany, where pioneering scientific knowledge and techniques of propagation were developed has been successful, among other things, in tissue culture propagation of bamboos. Plantlets have been transferred to the field for evaluation. The technique for tissue culture production of plantlets has been perfected for *Dendrocalamus strictus*. Laboratory techniques are being perfected for other species of bamboos as well.

At the Central Plantation Crops Research Institute (CPCRI) in Kasargod, the Department of Biotechnology has sponsored tissue culture of oilpalm and coconut. Efforts are on for raising clones of high yielding coconut and oilpalm through in-vitro somatic embryogenesis. Vegetative tissues were excised by the non-destructive methods from native oilpalm trees. Leaf explants have been cultured in-vitro.

The Jawaharlal Nehru University (JNU), New Delhi and the Tamil Nadu Agricultural University, Coimbatore are also engaged in raising clones of high yielding coconut through in-vitro somatic embryogenesis.

Bhabha Atomic Research Centre (BARC), Bombay is working for clonal propagation of oilpalm by using explants from adult plants. Experiments have also been conducted on root induction as well as establishment of plantlets in soil. About 100 plants have been transferred to soil.

The Indian Institute of Science, Bangalore has been engaged in research on plant tissue culture of sandal, rosewood and eucalyptus.

Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow is working on plant tissue culture of several medicinal plants. The MS University, Baroda, the Regional Research Laboratory, Jammu and the Banaras Hindu University, Banaras are also working in the field of medicinal plants.

University of Jodhpur, Rajasthan has been engaged in research on the tissue culture propagation of arid zone plants like *Prosopis, Terminalia phoenix, Acasia* sp., etc. Success of their efforts will have tremendous impact on the regeneration of green cover particularly through wasteland development.

The Indian Institute of Chemical Biology, Calcutta is pursuing studies on the process for enzymatic hydrolysis of homicellulosic agro-residues to formentable sugars.

International Crop Research Institute for the Semi-arid Tropics, Hyderabad presently is in the process of enlarging their biotechnology facility. It is already using molecular techniques to identify virus pathogens in crops and agrotoxins on groundnuts, and on gene transfer across sterility barriers in sorghum and groundnut.

In the private sector, Hindustan Lever is working on tissue culture propagation of coconut and oilpalm and on biological nitrogen fixation. They have discovered organic compounds which greatly increase photosynthesis in plants and have also succeeded in producing certain bioinsecticides.

B. MEDICAL BIOTECHNOLOGY

I: Vaccines

The production strategies for preventing childhood diseases and

certain preventable diseases are worked out through an existing Science & Technology Mission of the Government. The Mission is involved in planning for the production of DPT, BCG, typhoid, polio, measles, rabies and hepatitis-B vaccines. The Department of Biotechnology has set up Expert/Technical Committees to evaluate the state of the art technologies for the production of oral polio vacinne and inactivated polio vaccine. The Expert/Technical Committees have chosen the vero cell micro carrier fermentation technology for the production of both inactivated polio vaccine and the tissue culture rabies vaccine. The most advanced technology chosen for the production of hyper-attenuated measles vaccine is the chick embryo fibroplast cell culture technology. Similarly, for the production of oral polio vaccine, the primary monkey kidney cell culture based technology will be employed. Considerable progress has been achieved towards the establishment of two vaccine production units—one in the joint sector (for inactivated polio vaccine, rabies vaccine and measles vaccine) and the other in the public sector (for oral polio vaccine). The negotiations for transfer of these technologies with foreign collaborators are in advanced stage. It is expected that the indigenous bulk production of the above mentioned four vaccines will be realized by 1990-1991.

Several other diseases where vaccines need to be developed are malaria, tuberculosis, leprosy, diarrhoeal diseases and filariasis. Development of malaria vaccine would perhaps be a long term goal. The developmental efforts would have to be substantially strengthened in institutions devoted to R&D in these diseases. Since development in certain other countries has been more, collaborative efforts with those countries would be fruitful. Useful work has been done in this country towards the development of vaccines for leprosy. All round support would have to be provided to further pursue the work to convert this into a technology.

The Pasteur Institute of India, Coonoor, the National Institute of Immunology, New Delhi, the Indian Institute of Science, Bangalore, Madurai Kamaraj University, Madurai, All India Institute of Medical Sciences, New Delhi, National Institute of Virology, Pune, different centres of the Indian Council of Medical Research, Foundation of Leprosy Research, Institute of Cancer Research, Bombay and National Institute of Communicable Diseases, New Delhi are involved in the development of effective and reliable new vaccines based on r-DNA and Hybridoma techniques against tropical diseases like cholera, leprosy, tuberculosis, typhoid and diarrhoeal diseases. Some of these institutes like the National Institute of Immunology, are pursuing projects for the development of contraceptive vaccines using immunological approaches. The

infrastructural strength built up by these institutes provides scope for extending bilateral cooperation to other countries for vaccines against most of the communicable diseases.

II: Diagnostics

Several companies in the country have started making diagnostic kits by procuring them from abroad. These include early detection of pregnancy, detection of communicable diseases such as amoebiasis, typhoid fever, toxoplasmosis, hepatitis-B, AIDS, etc. and several non-communicable diseases like various forms of cancer, levels of certain enzymes, etc. There is strong need in the country to develop techniques and simple kits for the early detection of malaria, leprosy, tuberculosis, filariasis, leishmaniasis, diarrhoeal diseases, etc. These need to be simple, accurate, robust, appealing, convenience to use and must be reasonably priced. While the techniques for the detection of certain aliments have been perfected in certain laboratories in India, they need to concentrate on developing kits keeping in view the commercial angle also. Kits need to be developed by indigenous efforts even if they have already been developed abroad. Collaborative efforts at various levels would be encouraged if advantageous to India.

The Department of Biotechnology has initiated the S&T project entitled "Development and Production of Immunodiagnostics" to develop diagnostic kits for the detection of several commercial diseases, and early detection of pregnancy and other conditions of human reproduction. Several institutes are involved in the development of diagnostic kits for amoebiasis, leprosy, tuberculosis, filariasis, malaria, typhoid, hepatitis-B, rota virus, diarrhoeal diseases and respiratory tract diseases.

For developing simple, sensitive and reliable methods for early detection of physiological disorders, pathological and communicable diseases, immunodiagnostic kits based on ELISA technique are being attempted in various laboratories like the Central Drug Research Institute, Lucknow; National Institute of Communicable Diseases, New Delhi; Postgraduate Institute, Chandigarh; All India Institute of Medical Sciences, New Delhi; Institute of Research in Reproduction, Bombay; Mahatma Gandhi Institute of Medical Sciences, Wardha; Haffkine Institute for Training, Research and Testing, Bombay, etc. Kits for early detection of pregnancy, typhoid, amoebiasis, leprosy, tuberculosis and hepatitis-B are in various stages of development. The development of diagnostic kits for filariasis, toxoplasmosis, allergens, alphafeto proteins, gonadotropins, T-3, T-4 hormones, etc. has already made considerable progress. Efforts are being made to develop those already successfully tried into kits. The Institute of Microbial Technology,

Chandigarh is also developing a novel approach towards developing B and T cell specific immuno modulators. The Indian Institute of Chemical Biology is working on immunoassay techniques for the diagnosis of pathological conditions and therapy monitoring, as also for suitable drug delivery system. The Haffkine Institute for Training, Research and Testing, Bombay is working on the development of newer methods for diagnosis of communicable diseases of national importance. The institute is also working on the identification, isolation, characterization of immunogenics antigens typing of parasite isolates and clones, etc. The National Institute of Immunology, New Delhi; All India Institute of Medical Sciences, New Delhi; Central Drug Research Institue, Lucknow; Jawaharlal Nehru University, New Delhi; National Institute of Cholera and Enteric Diseases, Calcutta; Indian Institute of Chemical Biology, Calcutta; and Institute of Microbial Technology, Chandigarh are working on immunodiagnostics and DNA probes for tuberculosis, malaria, typhoid, diarrhoeal diseases, rabies, cancer, AIDS, prenatal diagnosis, etc.

III: Therapeutic Biomolecules

Technologies for the production of biomolecules having specific applications such as protein hormones, certain monoclonals and polyclonals, synthetic peptides, oligonucleotides and reagents for immunodiagnostic use need to be developed in India. In certain cases such technologies could also be imported. Typical products include production of insulin, growth hormones, FSH, hCG, Urokinase, IL-2, interferences, secretins, somatostatin, etc. produced by conventional methods as well as by application of recombinant DNA technology. Procurement or development of technologies for the production of monoclonals and polyclonals to specific antigens like MCH-1 and 2, T- and B-cell antigens, specific parasite antigens, etc. is also to be encouraged.

The National Institute of Immunology, New Delhi; the Jawaharlal Nehru University, New Delhi; Anna University, Madras; AIIMS, New Delhi are working on production of several biopeptides technology using various expression system. Work is being done on growth hormones, hCG, FSH, insulin and biopesticides. The National Institute of Immunology, New Delhi; AIIMS, New Delhi; Indian Cancer Research Institute, Bombay; Jawaharlal Nehru University, New Delhi; Foundation of Leprosy Research are working on production of monoclonals and polyclonals specific to antigens, e. g. human monoclonals against MCH-1 and MCH-2 antigens, T cell and B cell antigens, parasite antigens, etc. The Centre for Biochemicals, New Delhi and the Institute for Research in Reproduction, Bombay;

Centre for Cellular and Molecular Biology, Hyderabad are working on production of synthetic peptides, oligonucleotides and other reagents like biotin, streptavidin, alkaline phosphatase, beta-galactosidase, etc. The Institute of Microbial Technology, Chandigarh is engaged in molecular approach to control of rota virus pathogenesis. The Malaria Research Centre, New Delhi is conducting studies on various immunological aspects of malaria. The Centre also imparts training in cell culture work and provides biological materials to other investigators.

IV: Drug Delivery System

Work is being carried out in the Department of Biochemistry, University of Delhi for developing various targetted drug delivery system. A full-fledged centre for development of liposome mediated delivery system has been developed at the Department of Biochemistry, University of Delhi; the National Institute of Immunology, New Delhi; the Central Drug Research Institute, Lucknow; the Institute of Microbial Technology, Chandigarh; and the Institute of Chemical Biology, Calcutta are woking on various aspects of drug delivery system using liposome and other systems.

V: Industrial Biotechnology

Nearly 40 antibiotics are being consumed in the country, although only about 15 are being manufactured. Substantial production facilities have been set up for penicillins, streptomycin, tetracyclines, chromophenicol and erhthromycin. The current technologies are not up to the international standards in terms of plant size, productivity and product cost. The existing technologies need to be strengthened either by import of contemporary technologies or by putting substantial R&D effort to update them. All support needs to be given for such venture. As regards aminoacids, the entire requirement is currently met through bulk imports. Major consumption items are lysine, glutamic acid, aspartic acid, methionine, etc. Certain enzymes like diastase, papain, etc. are being produced to some extent. An important enzyme penicillin acylase required for the production of 6-APA is produced in the immobilized form by one company only, while others depend on import. Many other enzymes required in small quantities are procured through imports only. There is a need to strengthen the technological base of the country in several of these areas by import of contemporary technology. Simultaneously steps need to be taken to strengthen the R&D base in the country. The specific items in this regard are listed below.

a) Penicillin G/V 1st crystals
b) Gentamycin
c) Rifamycin/Rifampicin
d) Cephalosporin C
e) Clavulanic acid
f) Immolize penicillin G/V acylase
g) Lysine
h) Glutamic acid
i) Aspartic acid
j) Methionine

VI: R&D Institutions in Industrial Biotechnology/ Fermentations

The Biochemical Engineering Research Centre, Indian Institute of Technology, Delhi is working on different aspects of fermentation technology starting from strain improvement using mutagenes/genetic engineering right up to pilot plant development stage with computer control. The Centre has also worked on production of microbial oil from cane molasses, two-stage catalytic delignification process for agricultural residues, production of ethanol from cellulase hydrolysate in batch and continuous system employing free cells recycled and immobilized cells. Department of Microbiology, MS University, Baroda; National Chemical Laboratory, Pune; Jadavpur University, Calcutta; Regional Research Laboratory, Jammu; Regional Research Laboratory, Jorhat, CFTRI, Mysore; and Department of Microbiology, Panjab University, Chandigarh are some of the other important centres where experimentations in fermentation are being carried out. The Institute of Microbial Technology, Chandigarh is engaged in construction of improved strains by genetic manipulation and process optimization in industrial ethanol fermentation and genetic and biochemical approaches for improved process development in rifamycin-B fermentation. The National Chemical Laboratory, Pune has its Microbial Technology programme related to the following four major aspects: (i) source identification; (ii) basic biology and metabolism; (iii) molecular biology; and (iv) fermentation technology.

The Indian Institute of Technology, Kharagpur is working on solid state fermentation for biogas production, development of landfill bioreactor for methane production from solid wastes, and development of biological contactors for treatment of waste water.

Major centres in India where work on immobilized enzymes is going on include HAL, Pimpri; ITT, Delhi; NCL, Pune; Jadavpur University, Calcutta; and IICB, Calcutta. Anna University, Madras is working on industrial enzymes. The work being done covers immobilization of enzymes for use in medicines, industrial fermentation, food processing and waste water system.

Initiative of Industry: Work on fermentation technology is being actively pursued at Hindustan Antibiotics Ltd (HAL), Pimpri; Indian Drugs and Pharmaceuticals Ltd (IDPL), Rishikesh; Anil Starch Product Ltd, Ahmedabad; Bharat Serum and Vaccines, Thane; Cadila Laboratory, Ahmedabad; Hindustan Lever Ltd, Bombay; Hoechst Pharmaceuticals Ltd, Bombay; Sarabhai Group, Baroda; Alembic Chemicals Ltd, Baroda.

C. MICROBIAL CONVERSION OF WASTES

I: Agriculture, Forestry and Food Industry Wastes

Activities in agriculture, forestry and food industry generate large organic wastes some of which must be treated. Treatment of such wastes can also give rise to various products of economic significance. They can be degrated into fermentative compounds and transformed into proteins by microorganism. The cultivation of algae on waste water contributes to the purification and provides a biomass high in proteins. Large quantities of cereal straw (rice, wheat, etc.) and byproducts of cultivation of maize, bajra, pulses, etc. are generated in the country every year. Sugar industry produced large quantities of sugar cane stems, some surplus bagasse and large amounts of pressing residues. Numerous fruit juice production units generate large quantities of pulp, husks and residues which have no apparent use. Such byproducts are no doubt scattered and gathering costs are sometimes high. In many instances, however, the gathering costs are low and could be considered for use for producing value added products. Agricultural forestry and food wastes containing carbohydrates can be transformed by microbial fermentation into useful products. These wastes contain cellulose, hemicellulose and starch as the major degradation of cellulose and hemicellulosic substances for production of ethanol, further and phenols are considered interesting. Starch rich substances are already used for production of alcohol.

According to estimates by experts, 30% of petrochemicals required for the world could be produced by microbial conversion of cellulose. Considering the potential, genetic recombination technique will be utilized to isolate strains best adopted to these conversions and higher yields. Such work as transfer of cellulose and hemicellulose genes of, say, clostridium thermocellum which grows at 67-75 ℃ approx. to other species of clostridium to convert cellulose and hemicellulose into

ethanol, acetone, butanol, acetic acid, etc. would be intensified so as to have the advantage of reducing the distillation cost of alcohol and make the process economical. Certain bacteria such as *Zymomonas mobilis* ferments sugar twice as fast as yeast. Such species would be used to genome modification to produce engineered microorganisms to enable degradation of cellulose and simultaneously ferment sugars resulting from the degradation into alcohol.

India being an agricultural country, where large quantities of agricultural wastes would be generated, such work as mentioned above needs to be intensified and collaboration wherever feasible with other countries could be encouraged.

II: Industrial Wastes and Sewage Treatment

Aerobic as well as anaerobic microorganisms are already used for the purification of industrial wastes and sewage. Good results are obtained in processing wastes from alcohol industry, oil and cider works, milk and dairy industries, starch processing industry, etc. by anaerobic process where some methane is also produced. In many industries, combinations of anaerobic and aerobic processes are used to reduce the waste load faster.

Microbial strains could be isolated in order to control various forms of toxic pollutants, for example, to decompose phenols, chlorinated hydrocarbons, cyanides, detergents, plastic materials, etc. It is already known that certain pseudomonas microbes are known to modify a molecule which is then degraded by others. This co-metabolism phenomenon was shown to destroy parathion by two strains of pseudomonas. Often detoxification is the result of a series of chemical conversion such as phosphorilation, methylation, acetylation, etc. which takes place on chemicals engulfed by certain microbes. The enzymes that catalyse such reactions are often specified by genes borne by plasmids. Thus genetic recombination techniques should make such possibilities a reality to enable the mankind to produce engineered microbes for use for specific purposes. As such work is not being done currently in this country, it is time to take up such projects in appropriate institutes.

D. BIOPESTICIDES/INSECTICIDES

In recent years, increasing attention has been paid to the biological methods of control of insects which are responsible as vectors of diseases and destroyers of crops. Strains of *Bacillus sphaericas* are being tried for the control of mosquito vectors responsible for communicable diseases like malaria and filaria. *Bacillus thuringiensis* produces a toxic protein which kills

lepidopterous larvae in a few hours. The bacteria has a very wide host range among the caterpillars. *Bacillus popilliae* has been used to control the Japanese beetle. The two bacilli, namely *B. sphaericus* and *B. thuringiensis* could be grown in large quantities in pilot as well as commercial fermentors. It needs to be seen if the spores either living or dead could be used extensively for the control of certain vectors in the country. Like bacteria, several fungal spores germinate on the insect cuticle and penetrate directly through it to the tissue within. In other cases, the penetration takes place via the gut. Fungal growth invades the organs and kills the insect. Several fungi studies for this purpose include *Verticillium lecanii, Hirsutella thempsonii, Metarhizium anisopliae, Beauveria spp., Nomuraea rileyi, Culicinomycees clavisporus, Tolypocladium cylindrosporium,* etc. *V. lecanii* is a naturally occurring pathogen of aphids, white flies and scale insects. Spores are stated to be easily culturable on large scale. *M. anisophiliae* is reported to infest over 200 insect hosts. It has been used to control rhinoceros beetle and sugarcane pests. Mass production techniques have also been perfected elsewhere. *B. bassiana* and *B. brongniartic* are known to infect over 400 insect species. Spores are easily cultured in largescale. However, this fungus has not yet been extensively used world over. *C. clavisporus* and *T. cylindrosporum* are pathogens for mosquitos. Production methods of the spores are not difficult. These are being studied in several parts of the world; however work has not yet started in India. Identification of pathogens, characterization of the insect hosts, the knowledge about the specificity between pathogen and the host, the techniques of production, storage and application methods, etc. need to be acquired extensively in this country. In India, work has started a couple of years ago on the use of *B. sphaericus* and *B. thuringiensia* for the control of strains insects. The work needs to be expanded to use such strains for the control of many more insects.

E. ANIMAL HUSBANDRY AND AQUATIC LIFE FORMS

Technologies in the following areas need to be developed:

1. Products and processes based on embryo transfer technology and artificial insemination.
2. Transgenic life forms by hormonal and other treatment.
3. Monosex and sterile life forms by various techniques.
4. Food and other nutritional supplements for fast growth of poultry animals as well as aquatic life forms.
5. Methods for the preservation of embryos and gametes for large periods.

Specific R&D projects in the above areas are being identified

by the Department of Biotechnology in consultation with the Indian Council for Agricultural Research at various institutions in the country with a view to developing commercially useable products/processes. Collaborative efforts with other countries could be welcomed.

F. CONCLUDING REMARKS

For the development of economically usable technologies in any of the above areas, the scientists and technologists would have to offer creative solution for either introducing new capabilities or enhancing current efficiencies. The solutions provided would have to be complete so that there is overall satisfaction to the entrepreneurs, the production people as well as the consumers. In several areas, substantial basic work has already been done. In some instances, the basic work has progressed quite well and with more conserted efforts such work could be converted into usable products and processes. All round support is being provided to the institutes devoted to such work. Collaborative research in many such areas could also be undertaken with other laboratories in many other countries. Such joint collaboration will no doubt be mutually interesting and beneficial for both sides on a long term basis.