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Brazil. Centre for Biotechnology.

Ernst L. Winnacker.

14762

Report on a mission to Brasil
March 10 to March 23, 1984.

My trip to Brasil was planned to serve two purposes:

a) to perform a course in molecular biology techniques for interested staff from the FTI in Lorena (SP) and

b) to consult FTI on the extablishment of a Center of Biotechnology in Lorena (SP).

The course offered three types of experiments:

a) purification o. restriction enzymes;

b) plasmid purification and transformation using advanced technology expression vectors;

c) use of bacteriophage vectors and preparation of gene libraries.

In order to perform these experiments, the consultant brought to Brazil the necessary material, including literature, chemicals and various pieces of equipment (value 2.000 $). Most of the equipment was donated to FTI in Lorena, some was left with Prof. F. Lara, University of S. Paolo. Due to the lack of heavy equipment at the FTI laboratories in Lorena, the course was performed at the Department of Biochemistry, Univ. of S. Paolo (USP) in the laboratory of Prof. F. Lara. His excellent staff was instrumental to the success of this mission and participated actively in the course. Most experiments were adapted to the requirements and interests of the group of Prof. Lara.

The consultant gave introductory lectures on all the different experiments and their scientific background and discussed their actual progress with the participants. Most experiments could be concluded successfully until Saturday, March 17. The participants from Lorena included Mrs. Celia Schreiber, a technician with a Masters degree from the Univ. of Vienna, and Prof. Y. Levanon, a Prof. of FTI in Lorena.

The consultant was impressed by the high scientific standard in the laboratories of Prof. Lara and his collegues. These include, in

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particular, Prof. El-Dorry, a senior scientist in the the same department and previously Assistent Professor of Biochemistry at Cornell University, Ithaca, N.Y., USA.

The second part of the mission was performed in Lorena, SP, at the premises of FTI. Its objective was to provide a framework for future activities of FTI in the biotechnology aerea, in particular the planned Center of Biotechnology. The participants in the discussions during the four days included:

Dr. Paolo Afonso (FTI),

Mr. Carlos Urban, Mr. S. Kling and Mr. S. Caillaux,

Dr. C. Coulson, (FAO),

Mr. W. Kamel (UNIDO),

Prof. D. Ryu (UC Davis) and myself.

The panel reviewed the progress of the FTI efforts in the field of biotechnology. These are summarized in two papers attached as Appendices to this report. The UNIDO experts decided to propose to FTI a series of experiments which could be performed successfully within the next twelve months and which would permit an immediate start in the field of genetic engineering.

The mission was concluded with an extended discussion of the results with the directorate of FTI, in particular Prof. Juarez and Dr. P. Afonso.

During his two weeks mission in Brazil, the consultant had the opportunity to visit two excellent laboratories, the lab of Prof. Lara at USP and the laboratory of Prof. Morel at the Oswaldo Cruz Institute in Rio de Janeiro. Both places were adequately equipped. It became obvious in the course of these visits that there is an impressive potential of scientists in Brazil who are well educated and who can perform scientific work which is competitive with international standards. The chances therefore, to found the Center of Biotechnology of FTI appear quite promising.

Munich, April 10, 1984.

Ernst-L. Winnacker
Prof. of Biochemistry

RESEARCH PROPOSAL FOR THE CENTER OF BIOTECHNOLOGY

MARCH 1984

A) Background and accomplishments
B) Objectives
C) Research proposals
   a) Starch
   b) Microbiology
   c) Chemistry

A) Background and accomplishments.

The following research program outlined below is based on the following material:

a) The report of a panel of UNIDO experts from November 1983 discussing the goals for the Center of Biotechnology in Lorena, SP.; these goals basicaly center around biofuel production.
b) The "Guidelines for Present Future Activities of FTI" prepared by Dr. Y. Levanon.

c) A discussion between UNIDO experts and staff members of FTI in Lorena on March 20, 1984.

d) A report on "Activities Accomplished by Biotechnology Division" by Mr. S.G. Caillaux and Mr. S.H. Kling, Lorena, March 1984.

It is obvious from these various sources and was recognized by the international experts that the Biotechnology Division of FTI had been very successful in the past year. It had reached and surpassed its goals and with its 10 projects had laid the ground

for the future activities in this field. The experts recognized the progress during the past 3 months made in the areas of staffing and equipment. It was also appreciated to hear that the funds for the Center of Biotechnology have been guaranteed by STI and that plans have been initiated to set up a temporary structure for the CB. High priority is given by the FTI to the choice of a chief scientist and of high-level personnel in the field of genetic engineering, microbiology, biochemistry and chemistry. Thus the goals set by the director of FTI and UNIDO experts in November 1983 have been met successfully; a solid base has been provided for future work on the CB.

B) Objectives

Within the framework outlined above and in the source material it was proposed to define research projects with the following objectives in mind.

The projects should yield immediate results for the FTI within the next 12 months and, in addition, should have significance for the future work of FTI. The projects should be based on the existing R+D interests of FTI as outlined above; they will be designed to establish the FTI as a recognized Center of Biotechnology within the scientific and the scientific-political community; they should be realistic, practical and of immediate industrial interest. Some of the projects will be performed entirely in Lorena, some will require support from other laboratories in neighbouring institutions (e.g. USP). The projects certainly are within the scope of the existing UNDP/UNIDO/FAO - Program and will provide a solid frame work for future activities.

C) Research Programs

Three research programs will be presented which meet the conditions outlined above. They relate to the use of starch as biomass, to the microbial production of commercial chemicals (as outlined in pags. 3 and 4 of the "Guidelines"), and to the

chemistry of nucleic acids.

C.1. Starch degradation.

The use of starch as a renewable resource for glucose is of considerable practical interest. The complete enzymatic digestion of starch to glucose requires a variety of enzymes (amylases) which are produced by certain microorganisms. The genes for some of these enzymes, e.g. α-amylase, have been cloned from certain Bacillus strains. They should be transferred into other organisms (e.g E.coli and yeasts) in order to optimize their expression, and in order to permit these organisms to use starch as a carbon source. A prerequisite of this genetic engineering program are certain laboratory requirements which - at present - are not fulfilled in Lorena. This project thus would have to be performed in a laboratory which is adequately equipped. In addition, it would be paramount that this laboratory has already done preparatory works in the field. With respect to the starch-degradation project, the laboratories at USP appear ideal for such a collaboration. The FTI would gain considerably from such a collaboration, it would rapidly be able to publish in an important field which has high priority in R+D activities of the Brazilian government; it would train people who could build a nucleus for further activities of FTI in Lorena itself.

C.1.1 Research activities

The work would involve the identification of Bacillus strains which produce the enzyme in question. The corresponding genes would be cloned by established techniques; the genes would be transferred to expression vectors to study the expression in other organism, e.g certain yeast strains. The project is not new, but straight forward.

It would provide a comparatively easy and practical entry for FTI into the field of genetic engineering.

C.1.2 Personnel

The project would have to be performed with one Ph.D. (trained in this field) and one senior technician. For an initial 6 months period they would be stationed in the host laboratory. In the meantime, the laboratories in Lorena would be prepared to permit the people to return to Lorena. An additional technician should be hired upon return of the first 2 people.

C.1.3 Equipment

Lorena already has some equipment for work in the genetic engineering field. A laboratory should be set aside to receive the equipment with the necessary power requirements. This laboratory initially would be shared with the microbiology group (see below). Existing equipment should be made functional.

The following additional equipment should be made available to the group upon their return to Lorena.

- 2 electrophosesis power supplies à 300 v (~400$)
- 1 " " " à 3000 v (~2000$)
- 2 electrophosesis apparatus - horizontal - with combs etc. (~300$ each) (these could be built in a good workshop)
- 1 electrophosesis apparatus for DNA-sequencing (3000$) (could also be selfmade)
- 2 tabletop centrifuges (12 - 15.000rpm) for Eppendorf-tubes
- 3 waterbath with temperature regulation
- 1 New Brunswick shaker for flaskes up to 2ℓ (8000$)
- 5 Pipetman à 200 and 1000 ℓ (~100$ each)
- photolab equipment (simple) for X-ray development;
- ultracentrifuge (optional) with vertical rotor.

C.2. Microbiology laboratory

An immediate goal of FTI within the existing projects is the improvement of the alcohol fermentation process as well as a search for new organisms for the degradation of cellulose, hemicellulose, etc. This work requires extensive microbial expertise. It is thus proposed that FTI initiates and establishes a microbiology group within the Lorena facility. This group would have the following research objectives:

a) Establishment in Lorena of techniques for enrichment cultures of anaerobic and aerobic microorganisms.

b) Identification of substrates of potential interest for the CB. It is proposed, in particular, to search for (anaerobic) microorganisms capable of using hemicellulose and cellulose as carbon sources. The project will have to rely considerably on the resources of Brazil itself, e.g. the tropical rainforest.

c) Establishment of a lab-scale fermentation for n-butanol, based on existing methodology and experience. The necessary strains can be obtained through ATCC.

d) Isolation and selection of microorganisms (from soil) which produce surfactants. The products can be expected to be bio-degradable and this would be of considerable interest in many applications (lubricants, cosmetics, etc.)

These projects can be initiated this year and will yield immediate results. They all have long-range perpectives within the framework of FTI projects. Performing these short-term projects the group would gain expertise and experience and would be able to set additional goals for future work.

C.2.1 Personnel

One Ph.D microbiologist
One senior technician
One lower-level technician

C.2.2 Equipment

Basic microbiological equipment does exist in Lorena. Additional requirements are:

1 Laminar - flow hood

1 gas-chromatograph

2 incubators (37°C and 32°C)

2 temperature-controlled shakers

1 functional autoclave

C.3 Chemistry laboratory

As discussed and outlined previously by the UNIDO experts, the CB will also require chemical/biochemical facilities. These activities could be initiated immediately in Lorena itself. The subject of work to be proposed is the chemical synthesis of DNA. This field has numerous applications, e.g in the synthesis of genes and in in vitro mutagenesis. There is already demand for these activities in Brazil. Recent developments in the field have simplified the technical requirements and make it feasable to set this technology up in Lorena. The PTI-group would be the first and only one in South-America to be active in the field. Starting materials and the synthesized oligonucleotide would be distributed on a commercial basis.

C.3.1 Research project

Several approaches exist for the synthesis of oligonucleotides. The most advanced and feasable technology is a solid-phase phosphotriester approach using phosphit chemistry. This technique permits additions of one nucleotide to a growing chain in the course of 15 min. and yields oligonucleotides of a length of 30 or longer in high yields. The chemistry is simple and straightforward; the necessary compounds are stable and available in Brazil. The UNIDO consultant offers to train personnel either in Munich and/or in Lorena.

C.3.2 Person 1

1 Ph.D. chemist (or an advanced senior technician in chemistry

1 low-level technician

C.3.3 Equipment

	Costs ($)
1 Rotary evaporator	1.500,00
1 N_2- pressure tank	500,00
2 Glass-distillation equipment	500,00
1 thin-layer chromatography, and various chemicals, including acetonitril, PCl_3, thymidine, adenosin, guanosin, cytidine, morpholine, methylenechloride, n-hexane, acetic acid ethylester, tetrazol, triethylamine, silicagel, benzoylchloride, acetylchloride, trimethylsilylchloride etc.	3.500,-

EXECUTIVE SUMMARY OF THE CB SCIENTIFIC PROGRAM

The UNIDO experts propose a scientific program for the Center of Biotechnology which a) should yield results within the next 12 months and which b) can be regarded as a nucleus for future work of FTI. The program centers around three main aereas:

1) Polysaccharide degradation (starch/cellulose)
2) Sucrochemicals - fermentations
3) microbiology

These main aereas have been defined in the November 1983 meeting of UNIDO experts and FTI directors. Within this framework, the following subdivisions have been identified:

1) Genetic Engineering
2) Biochemistry
3) Chemistry
4) Bioengineering

The UNIDO expert realise the limitations with respect to time and expenditure. A choice of three different subjects was thus selected which are either performed entirely in Lorena itself or in collaboration with neighbouring institutions. The subjects are as follows:

1) Genetic engineering

It is proposed to send two people (1 Ph.D. and one technician) to USP to work on the cloning of α-amylases from Bacillus-strains. USP laboratories are well prepared for this task. FTI thus would gain easy access to modern technology which would be transferred back to Lorena after six months.

2) Microbiology

It is proposed that a microbiology group is initiated in Lorena itself. The group (Ph.D., one technician) should perform:

a) enrichment cultures for cellulose and hemicellulose degradation.
b) establish a lab-scale n-butanol fermentation
c) select microorganism for surfactant production

The group will have a nucleus for further work in the genetic improvement of alcohol production etc.

3 ~~Biotechnology~~ Chemistry

Modern biotechnology and biochemistry cannot be performed without a basis of DNA-chemistry. It is thus proposed that a small group be set up in existing facilities in Lorena. The approach taken will be a phosphotriester-phosphite-solid-phase approach. Products can be distributed on a commercial basis with FTI being identified as the first institute in South-America in this emerging field. It follows almost automatically that this group could extend its work into the production of restriction enzymes which also could be distributed commercially in South-America. All three projects need some additional equipment mentioned in the detailed work program.

The specific objectives of the Center of Biotechnology are:

1. A Center of excellence devoted to research and development in renewable resources based on biotechnology will be established for public benefit.

2. The Center will devote substantial share of its effort to train and educate many future generations of scientists and technologists, as well as to promote international and regional cooperation among many countries having similar interests.

3. The short term task is to develop new process technology for ethanol based on biomass, and

4. The Center will broaden its future activities as part of a longer range program in the areas of sucrobiochemicals, fine chemicals, and agro-chemicals that are based on renewable resources.

Cognizant of these objectives of the National Center of Biotechnology and of the rapid changes taking place in the area of genetic engineering related biotechnologies in the context of Brazilian situation, the senior technical staff at FTI and three UNIDO biotechnologies programs at the National Center of Biotechnology, Lorena, SP, and updated the programs. The results of the review study and updated programs are presented to Dr. Juarez, Director FTI.

In the area of Bioenergy Process Technology Development Program, a significance progress made by the FTI is noted and a speedy pilot plant demonstration of the technology utilizing lignocellulosics before March, 1985 is recommended.

The process variables involved in pretreatment, enzymatic hydrolysis of cellulosics, ethanol fermentation with cellulose hydrolisate, and ethanol recovery should be studied further in order to determine the optimal process conditions and design parameters.

The pilot plant equipments that could be changed, modified, or newly acquired are identified and recommended.

Preparation for the pilot plant demonstration should be one of high priority development endeavor during next 12-month period.

The laboratory equipments that are urgently needed for the parellel effort of process optimization were also reviewed, identified, and recommended for a speecy acquisition.

The requirement for the additional technical personnel was assessed ad recommended. The technical capabilities development programs for the existing staff were also formulated and recommended with a consideration of broader biotechnology programs including genetic engineering.

The urgent need for construction of a laboratory nameed "Biotechnology Center Annex I" was recognized, and a design of such a laboratory including genetic engineering laboratory, microbiology laboratory, Biochemistry laboratory, Analytical and Instrument laboratory, and Mini-fermentation pilot plant with highly instrumented and computerized laboratory was recommended.

BIOENERGY PROCESS TECHNOLOGY PROGRAM

The results of assessment study on the "biofuel from biomass" program, the technical personnel, and the research and development facilities indicate that they appear to have been adequate up to now, but they should immediately begin to strengthen their personnel capabilities by additions and training in order to complete their "first generation biofuel process technology" that can be commercialized during the "first phase" of the program.

During the first phase, major changes, modification, and renovations of the pilot plant facilities will have to be made in order to effectively utilize the existing equipments and at the same time minimize the capital expenditure.

During the "second phase" of the program starting June, 1985, an improved bioenergy process technology should be developed and the scope of the research and development programs should be broadened to include the biotechnology programs related to the high value fermentation products, fine chemicals, and other specialty chemicals like gene products as well as other sucrobiochemicals and agrochemicals. During this period, both the laboratory and pilot plant facilities will have to be further strengthened to meet the needs generated by the expanded biotechnology programs and the more advanced second generation bioenergy process technology.

In order to carry out these research and development programs very successfully, an expert consultant will be extremely useful in many ways. He will be able to effectively transfer and disseminate scientific information related to the program, acquire those research materials that are sometimes difficult to or it takes too long to acquire, coordinate the activities related to the improvement of personnel capabilities, and review or assess the programs as need arises.

BIOENERGY PROCESS TECHNOLOGY PROGRAM

There are three major areas in this program

I) Bioenergy from lignocellulosics

II) Improvement of bioenergy process technology from starch (i.e. cassava)

III) Improvement of process technology utilizing by-products from bioenergy processes

The major sources of lignocellulosics to be evaluated are:

1) Sugar cane bagasse

2) Napier grass

3) Eucalyptus

4) Others

I.1) Pretreatment Process

1) Establish the optimal "steam-explosion" pretreatment conditions.
2) Operating conditions of "steam-explosion" to be optimized are: temperature, pressure, pH, initial moisture content, catalyst, power input per unit mass, etc.
3) Determine the best conditions for hemicellulose and lignin extraction.
4) Scale-up of pretreatment process from laboratory scale to the pilot plant scale.
5) Some of the physical-chemical variables that are important to the pretreatment of lignocellulosics to be studied are:
   - Study the crystallinity effect on hydrolysis
   - Cellulase adsorption pattern and its effect on synergism
   - Effect of bulk density
   - Effect of specific surface area on hydrolysis
   - Degree of polymerization effect on hydrolysis
   - Undesirable components of lignocellulosics - its identification and its adverse effect on hydrolysis
   - Study the effects of pretreatment conditions - Temperature, pressure, pH, moisture, lignin content, power input per unit mass of lignocellulosics as power efficiency.

I.2) Enzymatic Hydrolysis

1) The parameters important to the hydrolysis of lignocellulosics will be optimized for both laboratory and pilot plant scale batch process. The reaction and operating conditions to be optmized are:
   - Cellulose substrate concentration
   - The ratio of cellulase/cellulose substrate
   - The ratio of beta-glucosidase/cellulase
   - Agitation and mixing condition
   - The air-water interface effect on enzyme stability
   - Hydrolysis temperature and pH
   - Hydrolysis time
2) The rate and reaction mechanisms involved in enzymatic hydrolysis of cellulose will be carefully studied and the results used for scale-up and process design.

I-3) Fermentation Process

1) The fermentation conditions for batch process will be optimized for ethanol production.

The fermentation conditions to be optimized are the process improvement to be made are:

- Temperature and pH
- Sugar concentration
- Other nutrients and inhibitors
- Study the effects of hydrolysate compositions on ethanol fermentation
- Improve yeast viability by using air supplement and sterols
- Increase ethanol concentration and ethanol yield
- Increased sugar (hydrolysate) concentration
- Use high density cell culture system - immobilized or flocculated cultures
- Study the feasibility of continuous or semi-continuous fermentation system
- Development of a less energy intensive process technology or that requires low energy input by cogeneration or heat recovery

2) Improved instrumentation using high pressure liquid chromatography and other on-line analyser system will be implemented for the future automatic control of fermentation process and for an increased ethanol productivity.

3) Fermentation process will be scaled up to a pilot plant scale process.

I.4) Other Related Process Technology

1) The microbial strains and the fermentation processes that are used for cellulase production will have to be evaluated periodically, and attempts shoulds be made to improve both the strain (by genetic engineering technique or otherwise) and the fermentation process.
2) Ethanol recovery process technology should also be evaluated periodically and attempts should be made to improve the process technology in terms of energy consumption and ethanol yield.

   Supercritical extraction (i.e. CO_2) and solvent extraction process will be evaluated.

II) Improvement of bioenergy process technology from starch

A significant improvement of process technology using cassava has been achieved in the area of milling, communition, continuous hydrolysis, and distillation. Pilot plant scale evaluation of this improved process technology is underway, and scale down of this process technology for the small scale operation is being developed for widely distributed small scale application.

III) Improvement of process technology utilizing by products from bioenergy processes

Conversion process for lignin, pentose, stillage, fibrous residues, and other by-products from bioenergy process to high value products will be developed.

PERSONNEL CAPABILITIES DEVELOPMENT

It is recommended that during the first phase both the research and development personnel should be strengthened by adding more technical people with graduate level training.

Number of additional personnel required are shown in table I. The profiles of these new recruits are:

(1) Research Section

* One microbiologist preferably with genetics training or experience
* One microbiologist with fermentation training or experience
* One biochemist preferably with enzymology training
* One chemist with analytical chemistry training
* One biochemical engineer with specialty in reaction engineering training

(2) Development Section

* Two chemical engineers preferably with biochemical engineering training
* One electronic engineer with process control background
* One engineer with computer science background
* One mechanical engineer with materials and construction orientation.

Depending upon the budget and scope of the second phase program these technical personnel could be increased proportionally - for example doubling or tripling would be desirable.

For personnel capabilities improvement, it is recommended that:

1. In-house seminar and workshop should be held 2-4 times a year, and intensive course work in the related subject area could be carried out with the help of experts/specialists.
2. As need arises, some selected personnel should have a research or training leave at a reputable academic or research organizations (for example, some institutions in U.S.).

GLT - Graduate level technician
MLT - Medium level technician
AT - Administrative technician
NSW - Nonskilled worker

TABLE 1

PERSONNEL ESTIMATE FOR THE TECHNICAL UNITS FOR FIRST YEAR AND BEYOND OF IMPLANTATION OF CENTER OF BIOTECHNOLOGY

	1st YEAR					BEYOND (+ 5 YEARS)				
	GLT	MLT	AT	NSW	TOTAL	GLT	MLT	AT	NSW	TOTAL
Genetic Engineering	2	-	-	-	2	8	4	2	2	16
Microbiology	4	-	-	-	4	4	5	1	2	12
Fermentation	5	3	1	1	10	16	14	2	4	36
Biochemistry	4	2	1	1	8	6	3	2	2	13
Pretreatment & Biomass Byproduct improvement	5	2	1	2	10	3	2	1	4	10
Engineering & Bioreactors	12	5	1	6	24	12	8	1	15	36
T O T A L	32	12	4	10	58	49	36	9	29	123

SUPPORTING PERSONNEL - NON-TECHNICAL UNITS

	1st PHASE 1st YEAR					BEYOND (+ 5 YEARS) 2nd PHASE				
	GLT	MLT	AT	NSW	TOTAL	GLT	MLT	AT	NSW	TOTAL
Informatics	1	1	2	-	4	4	4	4	-	12
Computering	1	1	1	-	3	4	4	2	-	10
Chem.Lab.of Analytical Support	3	4	1	2	10	5	9	2	5	21
Electronics and Instrumentation	1	1	-	-	2	1	4	-	-	5
Fine mechanics and glassblower	-	2	-	2	3	-	3	-	6	9
Maintenance and Utilities	1	2	-	2	5	1	2	-	6	9
Administrative support	2	4	5	4	15	3	5	6	5	19
T O T A L	9	16	9	10	44	18	33	14	24	89

TOTAL PERSONNEL FOR C.B.

	1st PHASE 1st YEAR					BEYOND + 5 YEARS				
	GLT	MLT	AT	NSW	TOTAL	GLT	MLT	AT	NSW	TOTAL
TECHNICAL UNITS	32	12	4	10	58	49	36	9	29	123
SUPORTING UNITS	9	16	9	10	44	18	33	14	24	89
T O T A L S	41	28	13	20	102	67	69	23	53	212

LABORATORY EQUIPMENTS

The laboratory equipments were surveyed (see the list attached), and only those additional equipments that they will need immediately are listed here for consideration. (See ~~Attachment 4).~~ Table 2)

* Refrigerated centrifuge (ie Sorvall)
* Autoclave for sterilization and microbilogical work
* Ethanol analyzer (enzymatic or other methods)
* Glucose analyzer (enzymatic or equivalent)
* Laboratory scale highly instrumented fermentor system with controls
* Colony counter
* Moisture analyzer
* Gel electrophoresis system

See table 3 for additional equipments required for the first phase research and development programs.

TABLE I

EQUIPMENT (RESEARCH LABORATORIES)

1) NATIONAL

	Equipments	Cr$ 10^6
01	Photomicroscope	4,4
03	Common microscopes	8,8
01	Freezer (-20ºC)	20,4
01	Deep-freezer (-80ºC)	7,3
03	Domestic refrigerators	1,5
01	Automatic recording spectrophotometer	43,8
04	Analytical scales	8,8
01	Water double still	2,2
02	100 ℓ Autoclaves	0,9
04	Potentiometers	4,4
02	Vacuum pumps	2,9
04	Small centrifuges	8,8
04	Metabolic baths	11,7
04	Voltage stabilizers	1,5
01	Automatic electric generator for emergencies	14,6
01	Small laboratory items and small equipment	27,7
04	Rotary shakers	17,7
03	Ice machines	6,6
01	Equipment for photography and film development	14,6
01	Packages of collector columns and resins for certain chromatographic analyses	6,0
		214,6

2) IMPORTED

	Equipments	
01	Ultracentrifuge with accessories	58,4
01	Preparatory centrifuge with accessories (refrigerated)	45,0

02	Set of bench fermenters	80,0
04	Rotary evaporators	40,0
01	Colony counter	5,8
	Packages of columns, fraction collectors, and resins for certain chromatographic analyses	26,6
		219,8

ADDITIONAL EQUIPMENT-NECESSARY NOT INCLUDED IN THE PROJECT PROPOSAL EQUIPMENT LIST

		Cr$	
	Word processor	12,000,000	
02	Electrophoresis (power supply)	2,500,000 (1st phase)	
01	Ultrasound sonicator	1,800,000	(2x)
02	Units - Fermenters with accessories (5/10 ℓ)	15,000,000	(2x)
	Deoinizer lab		
	Homogeneizer	16,000,000	

PILOT PLANT FACILITIES

The pilot plant utilities and facilities have been inspected (the list provided are attached). Based on the available information, the followings are recommended. (See ~~Attachment 5).~~ Table 3)

1. Water softening system and distilled water supply system should be installed for enzyme work as part of the pilot plant utilities.

2. As the first phase development endeavor, the existing facilities should be modified, changed, or renovated in order to convert the facilites to biofuel process pilot plant. This recommendation is based on the strategy of minimizing the capital expenditure and making best use of the existing facilities.

3. Holival milling equipments could be modified and changed so as to test the possibility of using it as compression milling process unit for pretreatment of lignocellulosics.

4. The continuous acid hydrolysis set up (Stake II System) should be tested for possibility of using it as the steam explosion process unit. With this unit mild acid treatment or other pretreatment process could be combined with the steam explosion process with minor modification.

5. Some parts of acid hydrolysis pilot plant - mainly the reactors and some control systems - and parts of starch hydrolysis equipments could be converted to and utilized as reactors for pretreatment and enzymatic hydrolysis of lignocellulosics.

6. The existing fermentation and distillation pilot plant facilities should be saved and used as multi-purpose biofuel process pilot plant with either starch or cellulosics feedstocks. For the second or next generation biofuel process technology, those pilot plant equipments should be upgraded or modified to accomodate implementation of advanced process technology

7. Major changes in pilot plant layout and interfacing

between different unit processes will have to be undertaken. Some redesign and rebuilding will be required for conversion of part of the pilot plant.

8. Some additional changes and modifications of pilot plant equipmens are required for effective utilization of by products from bioenergy process.

9. Additional changes and modifications of pilot plant equipments and facilities are listed in table 2.

TABLE 3 - EQUIPMENTS - PILOT PLANT (Modification & Changes)

. Starch degradation
  - Hydrolysis
    . Root pre-treatement equipment 84/7
    . Fibers separation equipments
      . rotary screen - 1
      . stationary screens - 1
      . centrifuge(vertical) - 2
      . centrifuge(horizontal) - 1
    } 85/1
    . special non obstructing heat-exchangery - 4
    . pulsing pump and cooker equipment - 1
    } 85/4
    . instrumentation sensors for temperature, pressure, level, flow, viscosity, pH, etc. ~~varion~~ 84/10
      valve actuatory and automatic flow plon and pressure valve - varion
      signal transducers for panel and control desk.
    . control panel and desk - 1) 84/12

. Celullosic and lignocellulosic material degradation
  - Pretreatment
    . Steam-explosion equipment-modification for batch and continous operation - 1 84/10
    . Two-roll mill - 1 84/2
  - Hydrolysis
    . Equipment for small scale enzymatic hydrolysis 84/2

- General ~~of~~ Fermentations Process

. Fermentations : batch equipments - modifications and increase facilities

cooling 84/7
agitation 84/10
aeration 85/1
pumps (transfer) 84/4
feeding 84/4

. Continuous small scale fermentation equipments 84/7

- Recovery and separation steps

. bench installation for extraction with CO_2 ~~(non-supercritical/supercritical se~~ under Super critical and non-Super critical conditions~~)~~ 84/12

. design and construction of a small scale steps unit for solvent extraction 84/12

. Stillage filtration separation equipment
. Fermentation of stillage recovery equipment) 84/9

Air compressor (new)) 84/4
Distillation 2.500 l/day

Design of Biotechnology Center Annex I
Laboratory

In view an urgent need for biotechnology laboratory space, the Biotechnology Center Annex I is designed (primarly layout) with the help of Dr. Carlos Urban. This laboratory will have the following space allocations:

Total space:	1.170 m^2
Office	240
Genetic Engineering lab.	170
Biochemistry lab.	150
Microbiology lab.	150
Analytical Instrument lab.	100
Fermentation (Mini-pilot plant)	140

This laboratory will have the following supporting facilities:

Media preparation room	2,5 m^2
Autoclave sterilizing room	20,0
Sterile transfer room	25,0
Cold room (~ 4^oC)	35,0
Constant temperature incubation rooms (20^o, 30^oC)	35,0
water treatment	
organic solvent storage	outside
Utilities support	the building

The utilities services include:

Steam compressed, air, vacuum, gas, cold water (filtered and softened), hot water, deionized water, distilled water, organic solvent lines (to fermentation recovery), and electricity (110, 220 volts).

21 March 1984

Dear Dr. Gomes,

Equipment, media, chemicals and supplies donated to Fundação de Tecnologia Industrial

I am pleased to advise you that my equipment, media, supplies and chemicals given on the attached (German list) apart from those marked as given to Professor F.Lara, are donated to your Foundation. These are for use within the Centre of Biotechnology Research & Development Programme.

I would advise that they be under the supervision of Dr. Y.Levanon for the use of Mrs. Célia Schreiber, who is aware of their proper use.

The chemicals provided are not standard ones but of special quality for molecular biology purposes. The micro-organisms supplied will need re-plating on a monthly basis to ensure that they remain viable.

I am looking forward to seeing that they assist you in the programme in your Centre in their specific areas of use.

Yours sincerely,

Ernst-L. Winnacker

Ernst-Ludwig Winnacker
UNIDO Consultant
to
Centre of Biotechnology

Encl. (1)

Dr. Paulo Afonso de Faria Gomes
Deputy Technical Director
Fundação de Tecnologia Industrial
Av. Capitão Messias Ribeiro, 625
12.600 Lorena, SP

Calciumchlorid . $2H_2O$		500 g	Eppendorfhütchen
Tris		500	gelbe Spitzen
EDTA		200	blaue Spitzen
SDS		100	Impfnadeln und -ösen
NaOH		100	Baby-Gel App. kompl.
Succrose		200	UV-Lampe
K_2HPO_4		200	Petrischalen
KH_2PO_4		200	Abimed p 200 und P1000
Yeast Extract		500	
Tryptone .	ca	500	
Bacto Agar		400	
pH-Papier	1-14 5-10		
Phenol		100	
Kaliumacetat		100	
tet, amp, Chloramphenikol	je	5	
Glycerin	ca	250 ml	
Lysozym		1	
Ammoniumsulfat		500	
Agarose		50	
Cellulose Phosphate	ca	250	
Hydroxylapatite	ca	50	
Streptomycin sulfat			
Ethidiumbromid			
X-Gal			
Iptg			
Tesaband			
Bromphenolblau			

Sequenzing pack
T4 DNA Ligase
Eca R I
Bal 31
mp 7
} Dr. Lara

Filterpapier 3MM und BA85 – with Dr. LARA

Dear Dr. Gomes,

I would be pleased to seek to arrange for Prof. Y. Levanon to stay in my laboratory at the University of Munich for a mutuallz convenient time. This presumably would be combined with his travel for FTI.

Yours Sincerely

Ernst-L. Winnacker
Prof.

Dr. Paulo Afonso de Faria Gomes
Deputy Technical Director
FTI
12600 Lorena, SP