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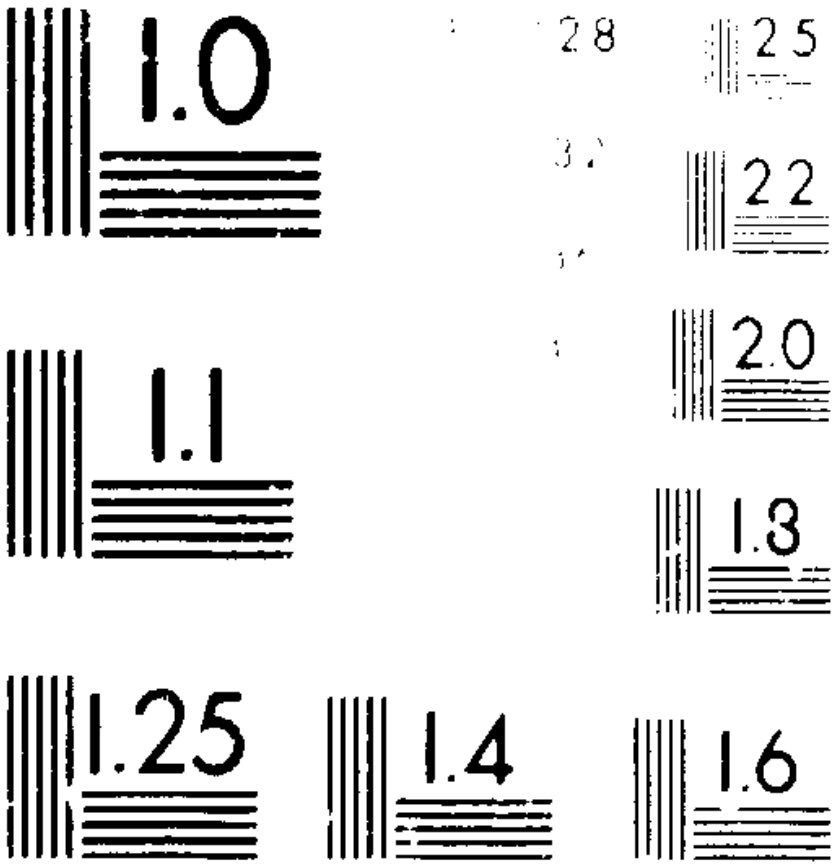

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INDIA

Technical Report*

Mission 25 February - 4 March 1984

Prepared for the Government of the Republic of India
by the United Nations Industrial Development Organization,
acting as executing agency for United Nations Development Programme

Based on the work of Peter P. Gray,
Consultant on Biotechnology
Conversion of Cellulose to Glucose

United Nations Industrial Development Organization
Vienna

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1. Project Background:

The aim of the project is to strengthen the expertise and research facilities at the National Chemical Laboratory (NCL) for the development of biotechnologies for exploitation of ligno-cellulosic resources and of technologies for controlled release pesticide formulation.

More specifically the project is aimed at development of fermentation process for the production of Microbial Biomass Product (MBP) from cellulose; development of enzymic process for hydrolysis of Cellulose to Glucose (CTG); development of a process for conversion of Glucose to Ethanol based on immobilized microbial whole cells (GTE).

The NCL has identified promising cellulolytic fungi (Penicillium funiculosum, Sclerotium rolfsii, etc.) on which laboratory studies are in progress towards optimizing fermentation parameters for high cellulase activities and productivities. Screening for hypercellulolytic mutants has also been initiated for Penicillium funiculosum and work done on the optimization of growth and enzyme production. Earlier studies on the microbial biomass product (MBP) using separate fungal cultures for the fermentation of cellulose to microbial biomass and for enzyme production have been temporarily discontinued from July 1983 on the advice of the non-resident Chief Technical Adviser, Dr. V.R. Srinivasan.

2. Purpose of the current visit:

The purpose of the current visit was to advise on the production of cellulolytic enzymes in high yields by submerged fermentation and the practical applications of such enzyme preparations for saccharification of agricultural and forest residues to fermentable sugar (including methods for substrate pretreatment, enzyme reuse, etc.); and to discuss programs on screening for hyper-cellulolytic mutants.

3. Comments on experimental program:

The progress on the experimental program has been described in some detail in the technical reports of V.R. Srinivasan dated 2nd July, 1983 and 31st August, 1982, and the reports to UNDP for the period March 1983 to August 1983 and preceding reports. Where necessary results obtained since the most recent UNDP report will be mentioned.

(1) Conversion of Cellulose to Glucose (CTG)

(a) As previously mentioned work on this program has focussed on Penicillium funiculosum both with respect to the rapid production of biomass and the production of high levels of cellulase enzymes and in the area of selecting hyperproducing mutants. In this later area the fact that colonies of Penicillium do not spread excessively over agar plates, a problem experienced with selecting for Trichoderma reesei mutants, has assisted in the isolation of higher yielding mutants. One of these UV-49 has shown the ability to produce 50% higher cellulase activities than the parent strain. Of particular interest is the high β-glucosidase activities of these strains, as one of the main problems with the Trichoderma reesei complex in its low β-glucosidase activity. Considerable work has been carried out on optimizing the conditions for enzyme production in stirred fermentors. With respect to maximizing cellulase enzyme

production, the NCL workers have reached similar conclusions to several other groups, i.e. that it is necessary to feed the cellulosic substrate to the fermentation at a rate which minimises catabolite repression effects. Accordingly they have arranged to have built a device which will allow the controlled addition of pelleted cellulosic substrate to the fermentor. It is anticipated that with this arrangement it will be possible to run fed-batch fermentations and to get considerably increased levels of extracellular protein and enzyme activities. This aspect of the program seems to be going well, particularly considering the relatively small number of people involved, although it may be necessary to use different mutagenic agents or screening approaches if it becomes hard to obtain improved mutants in the current mutation program.

(b) Several different pretreatment procedures have been used in the work including alkali, high temperature steam and ethylene diamine. As the pretreatment is a critical part of any cellulose bioconversion process the effectiveness and economics of any pretreatment process need to be closely studied. It would be useful to apply the considerable chemical engineering and process costing expertise existing at NCL Pune to determine the most cost effective pretreatment, e.g. conditions reported in Rao et al., Biotech. Bioeng., 25, 1863-1871 (1983) of 0.3g NaOH to 1.0g NaOH/g of sugar cane bagasse are way above any economically feasible levels and also use of ethylene diamine needs to be assessed as economically viable. If it were desired to set up in house high-temperature autohydrolysis pretreatments, then it might be possible to adapt for this purpose the vessels previously used for pulping, located on the mezzanine floor of the pilot plant containing the 10 l immobilised yeast reactor. It is worth noting that a cost effective pretreatment might also be of interest in India for the pretreatment of roughage for ruminant feeding.

(c) It appears that the program has suffered considerably from delays in getting the equipment provided by the UNDP. All the small scale equipment and fermentors are now in place and operational. The 100 l Chemap fermentor was being mounted on a plinth during the visit and building alterations continuing on the laboratory and control room around it. A serious problem at NCL in running fermentations, which often last up to 7 days, is the frequent interruption in the power supply. From the viewpoint of maintaining agitation and control to the vessels, the back-up power supply which exists at NCL and usually acts within 10 seconds of a power interruption is probably sufficient. However any disruptions/spikes in the supply will probably upset the microcomputer purchased to control the Chemap fermentor. Accordingly it is recommended that an uninterrupted power supply be purchased to operate the microcomputer and any other sophisticated control equipment.

4. General Comments:

The NCL Pune has an enviable record in R and D and technology transfer to Indian industry, particularly in the chemical area. One of the strengths of the biotechnology program at NCL is the presence of a true multi-disciplinary program with integration of the activities of microbiologists, biochemists, biochemical and process engineers. With this grouping it should be possible to have considerable impact on industrial applications of biotechnology in India. The UNDP program is supporting applied research in specific areas and it would seem that there should be more emphasis on certain areas such as strain improvement by mutation/selection and more sophisticated genetic techniques and less on the more basic biochemistry/enzymology of the cellulase enzyme complex. There has been considerable success at NCL in the isolation of microorganisms with novel enzymatic activities. It is felt that if the strain improvement area could be further strengthened this, coupled with the fermentation facilities provided by UNDP and the basic skills existing at Pune, would make for a group well suited to developing biotechnological processes for utilization in India.