OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

This document has been produced without formal United Nations editing. The designations employed and the presentation of the material in this document do not imply the expression of any opinion whatsoever on the part of the Secretariat of the United Nations Industrial Development Organization (UNIDO) concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries, or its economic system or degree of development. Designations such as "developed", "industrialized" and "developing" are intended for statistical convenience and do not necessarily express a judgment about the stage reached by a particular country or area in the development process. Mention of firm names or commercial products does not constitute an endorsement by UNIDO.

FAIR USE POLICY

Any part of this publication may be quoted and referenced for educational and research purposes without additional permission from UNIDO. However, those who make use of quoting and referencing this publication are requested to follow the Fair Use Policy of giving due credit to UNIDO.

CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

14803

RESTRICTED

February 1985
ENGLISH

India.

BIOSCIENCE AND ENGINEERING

DP/IND/80/003

INDIA

Technical Report *

Mission 1 December to 16 December 1984

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

Based on the work of Mary Mandels
Consultant on Biotechnology Conversion of Cellulose to Glucose

United Nations Industrial Development Organization
Vienna

* This document has been reproduced without formal editing.

Table of Contents

Abstract

I found the staff at the National Chemical Laboratory to be competent and well motivated. The number and quality of publications is impressive. Equipment is excellent, modern, and in good working order. The development of fundamental information on the cellulase systems of Penicillium funiculosum and Sclerotium rolfsii is a notable accomplishment.

I had useful discussions with various groups in Biotechnology, Biochemistry, Microbiology, Plant Cell Culture, and Biochemical Engineering. All were must helpful and open in discussing their work and in providing me with reprints and publications of their research.

Round table discussions on enzyme production, strain improvement, saccharification, and economic problems were held with staff members. I presented two public lectures, one on "Cellulase Enzyme Production," and one on "Saccharification of Cellulose with Trichoderma Cellulase." Achievements and problems still to be solved were emphasized.

My recommendations for the NCL program are that the work should focus on evaluation of a practical process. This effort should be coordinated so that a preliminary economic evaluation can be completed by the termination of the present phase (August 1986). This will require close collaboration by Biochemical Engineers, Microbiologists, and Biochemists.

Although some basic problems must still be solved before the economics of cellulose conversion are satisfactory, the prognosis is excellent that success will finally be achieved.

Project Background

The Project DP/IND/80/003 was requested by the government of India with the overall objective to strengthen the expertise and research facilities available to the National Chemical Laboratory (NCL) in Biotechnology of Renewable Resources for the production of food, fuel, and chemicals and in the technology of controlled release pesticide formulation. The proposal was developed as a five-year project (September 1981 - August 1986) under the support of UNDP, the government of India, and UNIDO with the following immediate objectives that are relevant to this visit.

a. Development of a fermentation process for the production of microbial biomass from cellulose.

b. Development of a process for the enzymatic hydrolysis of cellulose to glucose.

c. Development of a process for the conversion of glucose to ethanol based upon immobilized cell reactors.

The National Chemical Laboratories had developed some expertise in the biotechnology of biomass utilization, cellulose conversion, and ethanol fermentation before the implementation of this project. Initial support for these investigations came mainly from NCL funds and a small grant from FAO.

On the recommendation of Dr. V. R. Srinivasan, Chief Technical Advisor, the projects for production of biomass and for conversion of cellulose to glucose were combined in a single objective in July 1983, i.e. optimization of the growth of Penicillium funiculosum on cellulose and investigation of simultaneous production of cellulolytic enzymes from this biomass.

Purpose of the Current Visit

I was requested to advise on the production of cellulolytic enzymes in high yields by submerged fermentation and the practical application of such enzyme preparations for saccharification of agricultural and forest residues to fermentable sugars (including methods for substrate pretreatments, enzyme reuse, etc.), and to discuss programs for hyper-cellulolytic/constitutive mutants.

In addition, Mr. Maung requested my assessment of the present status of the economics of cellulose conversion to ethanol.

Economic Status of the Cellulose to Ethanol Process

Cellulose is an abundant renewable resource that can be efficiently hydrolyzed to glucose by microbial enzymes. The glucose, which retains the chemical energy of the cellulose, is a simple, soluble, stable molecule, easily separated from the digest, and useful for human or animal food or for production of chemicals. For example, glucose can be fermented to ethanol by yeast. Recently, there has been world wide interest in the use of ethanol, produced from biomass, as a liquid fuel to partially replace gasoline for the internal combustion engine. Alternatively, ethanol can be converted to ethylene, an important chemical feedstock. In Brazil, ethanol is produced in large quantity from sugar cane molasses and active research is aimed at development of processes for production of ethanol from cassava (starch) and from cellulose. In the United States, ethanol is produced from grain (starch). In both countries the ethanol is used to produce gasohol, a blend

of ethanol and gasoline. The use of edible carbohydrates (sucrose and starch) to produce fuel tends to increase food prices, and to reduce food exports, and is morally objectionable. Cellulose is a more desirable substrate, but is much more difficult to hydrolyze than is starch. Each of these substrates has its own specific enzyme complex.

Large quantities of glucose are produced commercially from starch by enzymatic hydrolysis. Despite generous research support, and many significant technical advances in enzyme production, pretreatment, evaluation of substrates, and utilization of by-products, no commercial process exists today for enzymatic hydrolysis of cellulose. The problem is economics, specifically the high enzyme requirement. It takes 100 times as much enzyme protein to produce a kilogram of glucose from cellulose as it does to produce a kilogram of glucose from starch. This is due to the greater recalcitrance of cellulose and the necessity for synergistic action of the several enzymes in the cellulase complex to achieve its hydrolysis. Because of this, there has been an active search for new strains and mutants which produce enzymes of higher specific activities and greater efficiencies, and for more effective pretreatments which increase the susceptibility of cellulose. Other means of decreasing the enzyme requirement include attempts to stabilize and recover enzymes from spent digests, and studies of enzyme substrate interactions to better understand the nature of enzyme synergism. Despite a consensus among knowledgeable scientists that a commercially feasible process for enzymatic hydrolysis of cellulose will eventually be developed, this will not occur until some progress is achieved on the above problems.

Comments on NCL Experimental Programs

During my stay at NCL, I visited laboratories in Biotechnology, Biochemistry, Microbiology, Biochemical Engineering, and Plant Cell Culture and discussed ongoing research programs with appropriate staff members. Everyone was most helpful and open in discussing their work and in providing me with reports and articles. In several cases, I was also given articles "in preparation" to read. Two two-hour round tables were held to discuss enzyme production, strain selection and improvement, saccharification, and economic problems with NCL staff members. I also presented two one-hour public lectures emphasizing the work done at Natick and elsewhere with Trichoderma reesei cellulase. At the request of the Acting Director, Biochemistry Division, I prepared a "Memorandum for the Director" outlining my findings and recommendations. This was presented to and discussed with the Acting Director NCL. Finally, a board meeting was held with the Acting Director NCL and principal NCL scientists and administrators to discuss the overall program and to answer any last inquiries.

The principal scientists with whom I held discussions and the subjects discussed were:

a. Dr. R. B. Mitra. Acting Director, NCL. General.

b. Dr. M. C. Srinivasan. Acting Director, Biochemistry Division, 1984. Overall programs and plans. Microbiology. Penicillium funiculosum studies. Saccharification. Enzyme Recovery. Direct Conversion of cellulose to ethanol with Neurospora crassa. Fusarium lini studies (cellulase). Penicillium

janthinellum studies (Production of Biomass). Also Dr. Vasanti Deshpande, Dr. Mala Rao, and Dr. Chitra Mishra in this group.

c. Dr. John Barnabas. Biochemistry. Genetics.

d. Dr. V. Jagannathan (retired). Director, Biochemistry Division until 1981. Penicillium funiculosum studies. Economics and outlook.

e. Dr. A. C. Manchandra. Biochemical Engineering. Ethanol Production. Also Mrs. Dr. H. Sivaraman, Miss A. V. Joglekar, and Mr. A. P. Pendre in this group.

f. Dr. J. C. Sadana (retired). Director, Biochemistry Division 1981-1982. Sclerotium rolfsii studies. Also Dr. M. C. Deshpande, Mr. Anil Lachke, and Dr. R. V. Padil in this group.

g. Dr. C. Sivaraman (retired). Director, Biochemistry Division, 1982-1983. Ethanol Production.

h. Dr. Karl Erik Eriksson. Swedish Forest Products Laboratory. Visiting Pune on a similar mission to mine. His last day coincided with my first day there.

The staff at NCL is well trained, motivated, and competent. Many of them have worked or studied abroad in well known laboratories, and they have active ongoing interaction with their international colleagues in cellulose conversion. They are active participants in international meetings and symposia, and they entertain frequent scientific visitors who come to NCL as consultants from UNIDO or on their own. In 1984, a number of scientists attending the International Biotechnology Congress in Delhi also visited Pune. Last year,

the Biochemistry Division published 35 papers from a group of 25-30 scientists. Most of these papers appeared in international refereed journals of good repute, were of high quality, and are widely read. The NCL development of fundamental information on the cellulase systems of Penicillium funiculosum and Sclerotium rolfsii is a notable accomplishment. Both of these strains produce a complete cellulase of good activity with high levels of cellobiase and hemicellulase. Recent work has demonstrated very high recovery of cellulase from hydrolysis syrups based on use of a high enzyme to substrate ratio achieved by adding substrate over time as previously added substrate is digested, and on grinding residues with glass beads to release adsorbed enzyme. Preliminary work on direct conversion of cellulose to ethanol by Neurospora crassa is very encouraging. This fungus shows greater ethanol tolerance than does Clostridium thermocellum the anaerobic bacterium being developed for direct conversion by the Massachusetts Institute of Technology group.

The chief problem at NCL is a diffusion of the effort over a number of projects and a lack of coordination of these projects towards a unified achievable goal. This is partly due to the sincere effort of the staff to respond to the various suggestions of the UNIDO consultants and perhaps also to frequent recent changes in the Director of the Biochemistry Division. There is not enough close cooperation between the microbiologists and biochemists who are mostly involved with fundamental cellulase studies, and the biochemical engineers who are mostly involved with the ethanol project. The consolidation of the fungal biomass project with the cellulose conversion

project disturbs them because the two goals are not compatible. Cellulase is a secondary metabolite and its production is repressed under optimum growth conditions. All enhanced cellulase mutants have reduced specific growth rates.

Recommendations

The work at NCL should focus on an economic evaluation of a practical process. This effort should be coordinated so that a preliminary economic evaluation can be completed by the termination of the present phase (August 1986). This will require close collaboration by Biochemical Engineers, Microbiologists, and Biochemists.

The NCL group should select a single cellulase source for development of a practical process based on specific activity and composition of the enzyme complex and its performance under process conditions and on enzyme productivity in large scale fermentation. As noted above, they have done outstanding work in developing information on the cellulases of Penicillium funiculosum and Sclerotium rolfsii. These cellulases are high in endo-β-glucanase, cellobiase, and hemicellulase. Most process development elsewhere has used cellulase from Trichoderma reesei. This cellulase is high in endo- and exo-β-glucanases, but deficient in cellobiase. Very high yielding mutants have been developed. Commercial cellulases are available from Penicillium funiculosum and from Trichoderma reesei. Enzymes from the three strains should be evaluated under use conditions (15% substrate, 50% conversion) on appropriate process substrates. I have supplied the NCL with a commercial sample of Penicillium funiculosum cellulase and a Natick sample of Trichoderma reesei

cellulase adequate for this evaluation and a model protocol for such an evaluation. If the enzymes are similar in specific activities, then fermentation yields in enzyme units per liter per hour may be the deciding factor. The economics of adding supplemental cellobiase from another source must also be considered. Since cellobiase has a much greater specific activity than cellulase, it is much cheaper, per unit, to produce. One cellobiase unit can replace one cellulase unit up to 50% of the enzyme mixture, i.e. 10 cellulase units plus 10 cellobiase units will equal 20 cellulase units in saccharification.

The ethanol project should be more closely coordinated with the cellulase work. As soon as possible, hydrolysis syrups should replace molasses in some of the ethanol work.

Enzyme production in the 15 liter New Brunswick Fermentors should be a major effort because optimization of the fermentation requires pH and other controls, and large quantities of enzyme will be required for evaluation of enzyme substrates and pretreatments and for production of hydrolysis syrups.

The promising studies on enzyme recovery from hydrolysis residues and on direct conversion of cellulose to ethanol by Neurospora crassa should continue as secondary projects.

As noted above, one cannot achieve both biomass and cellulase enzymes in a single process. The requirement to do this is distressing to the NCL staff.

The purpose of the suggested preliminary economic evaluation is to identify critical research problems. A discouraging evaluation should not

reduce support for the project. No cellulose conversion process is economically feasible today without some subsidy. However, the bottlenecks are not insurmountable. The oil crisis of yesterday has become todays' oil glut, but a new oil crisis is likely to develop in the not too distant future. A country like India is vulnerable to future oil shortages due either to political events or genuine depletion of a non-renewable resource.

Acknowledgement

I am most grateful to UNIDO for the opportunity to visit NCL and for the kind hospitality shown me there by the entire staff.