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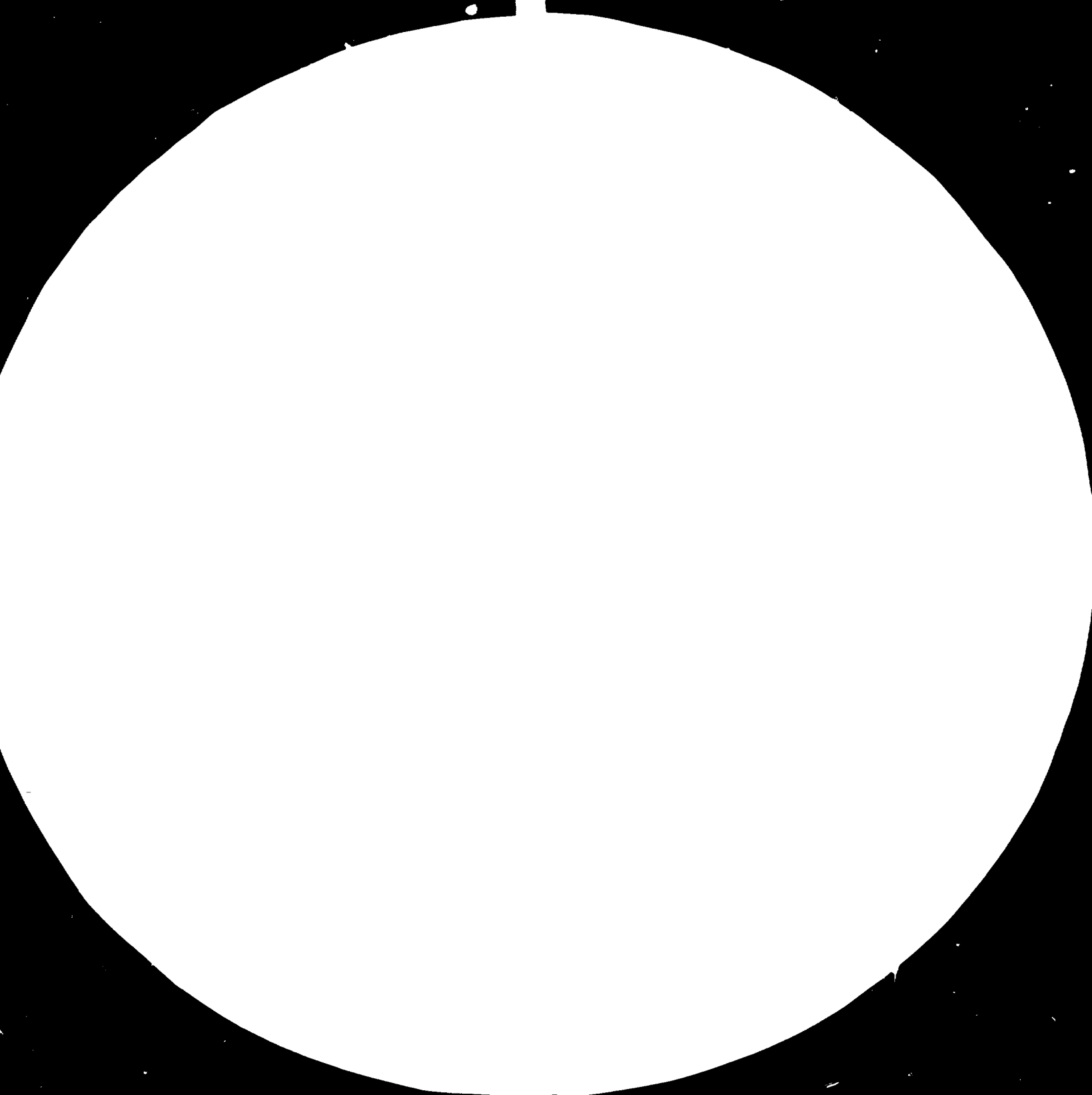
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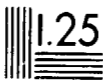
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MICROCOPY RESOLUTION TEST CHART

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BIOSCIENCE AND ENGINEERING .

DP/IND/80/003

Technical Report *

(Mission 22 July to 1 August 1984)

Based on the work of V.R. Srinivasan
consultant in fermentation technology

Chief Technical Adviser

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ABSTRACT

Investigations carried out during the period from July 83 - Aug 84 under the project Bioscience and Engineering DP/IND/80/003 were reviewed. Recommendations for studies for next year are outlined in this document. There has been satisfactory progress in optimization of biomass production and enzyme release on a laboratory scale. Production of ethanol by immobilized cells encountered a set-back during the pre-pilot investigations, and intensive and accelerated laboratory studies are in progress to regain some of the lost progress. The breakdown of the list of publications appended is as follows:

Number of papers published - 4

Number of papers accepted

for publication - 4

Number of abstracts of papers

presented in national and

international meetings - 10

1. Project Background: The project DP/IND/80/003 was requested by the government of India with the over-all objective to strengthen the expertise and research facilities available at the National Chemical Laboratories (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 with the following immediate objectives:

- (i) Development of a fermentation process for the production of microbial biomass product from cellulose.
- (ii) Development of a process for the enzymatic hydrolysis of cellulose to glucose.
- (iii) Development of a process for the conversion of glucose to ethanol based upon immobilized microbial cell reactors.
- (iv) Development of processes for the production of controlled release pesticides by microencapsulation and monolithic and matrix binding.

National Chemical Laboratories has developed a certain expertise on the biotechnology of biomass utilization and ethanol fermentation even before the implementation of this project. Initial support for these investigations came mainly from NCL funds; however, a small project support was obtained through FAO. Preliminary investigations on the CR formulation project were entirely supported through NCL funds.

2. Official Arrangements: The revised project document submitted as a project of the government of India in June, 1981 was approved by UNIDO and implemented in September, 1981. The duration of the project will be from September, 1981 through August, 1986.

On my recommendation in July '83, a midcourse correction of the project was carried out. The first two objectives were modified to be a single objective of optimization of production of biomass and enzyme in the same system. Thus the emphasis was shifted to optimization of growth of Penicillium funiculosum and investigations on the release of enzymes from the mycelium for the production of glucose from lignocellulosics.

3. Purpose of the present mission: The primary objectives of the present visit were to participate in the mid-term tripartite review of the project and advise on the further course of research and development of work in the areas related to microbial biomass product enzymatic hydrolysis of cellulose to glucose and production of ethanol from molasses by immobilized yeast cells.

In addition, the following lecture titled "Microbial Degradation of Lignin - Cloning of a gene for aryletherase" was presented to the members of the National Chemical Laboratory.

Besides, I had the opportunity to participate in the Second Project Advisory Committee meeting held on July 27, 1984.

4. Review of the present state of studies: Initially, a preliminary discussion was held with the Director of NCL about the over-all progress of the project. Thereafter, the investigations carried out during the period Aug. 83 - July 84 were discussed extensively not only with group leaders but also with individual investigators who actually performed the experiments. Furthermore, as it was done in the previous year, a separate meeting was held with the group leaders of the project to formulate the program for the following year and also to set target dates for several of the investigations. Results of these discussions are

summarized in the following with my comments.

Optimization of growth of Penicillium funiculosum and enzyme production.

- (i) Growth was optimized in 14 l. fermentors (10 l. working volume on glucose and cellulose as substrates. Cellulosic substrates were pretreated either with alkali or ethylene diamine.
- (ii) Experiments showed that doubling the concentration of cellulose as carbon source from 0.5% to 1% during fermentation doubled the biomass and cellulase activity.
- (iii) 50% of the carboxymethylcellulase (CMCase) 100% of the cellobiohydrolase (CI) and 30% of β glucosidase activities present in the mycelium are released by simple extraction with buffer.

Comments:

- (i) Fed-batch experiments showed that at least medium may not require any more optimization.
- (ii) There seems to be variability in growth experiments using ethylene diamine treated straw; however ethylene diamine treatment does not seem to affect the enzymatic hydrolysis of cellulose.
- (iii) Analyses of the growth and enzyme production leads to a preliminary indication that enzyme production seems to be highly regulated in this organism and is growth associated.

Conversion of Glucose/cane molasses to ethanol:

- (i) Scaling-up of laboratory studies on the production of alcohol by immobilized yeast on a high porosity matrix proved to be difficult.
- (ii) Scaling-up was done in a packed bed reactor (10-20 l/d alcohol) Difficulties due to gassing and disintegration of beads were encountered.
- (iii) The project was brought back to the laboratory. After a series of experiments on different matrices for immobilization, alginate seems to be the present choice for immobilization matrix.
- (iv) Studies on the selection of temperature-tolerant and salt-tolerant yeasts are in progress.
- (v) Based upon the experiments on a pre-pilot scale with packed bed reactor, studies are planned to experiment with a fluidized bed reactor.
- (vi) Equipment and chemicals for a 50-100 l/d alcohol production have already been procured.

Comments:

Although there was a set-back in the studies on pre-pilot plant (10 - 20 l/d alcohol production) valuable information has been obtained. There seems to be much better coordination between biochemists and engineers at present and also there is a keen sense of enthusiasm to bring the project to a successful completion.

Recommendations:

- (i) Since initial experiments showed that cellulase production seems to be associated with growth it is recommended that these experiments may be extended to two more series of experiments: a) with

2% alkali treated substrate in New Brunswick fermenters (14 l capacity); b) with 4% substrate in 150 l fermenter. These experiments may be completed by Dec. 1984.

- (ii) Releasing the enzymes from the biomass produced under optimal conditions, determine the maximum amount of pre-treated cellulose that can be saccharified. In these experiments compare the effect of alkali pre-treatment vs. ethylene diamine pre-treatment. Target date is April 1985.
- (iii) A preliminary economic assessment of the growth and saccharification process may be undertaken by Jan.-March 1985. This may include: a) alkali pre-treatment of the substrate for growth and saccharification; b) alkali pre-treatment for growth and ethylene diamine pre-treatment for saccharification.
- (iv) As recommended by other consultants, studies on the isolation of hyperactive strains be continued. Emphasis may be given to the two following approaches: a) Isolation of mutants with de-regulation in the production of cellulose enzymes; b) Initiate experiments on the molecular cloning of the genes for cellulase. (Details of experimentation were discussed in length with the investigators.)
- (v) Modified 50 - 100 L ethanol/day reactor to be in place by December 1984.
- (vi) Laboratory studies to determine probable cause of reduced ethanol concentration during continuous fermentation; whether wild yeast infection or intrinsic aging phenomenon (December 1984)

- (vii) Laboratory studies on matrices of Ca-alginate, CM-cellulose - polyacrylamide (December 1984).
- (viii) Pre-pilot scale studies based on results of 2 and 3 until (March 1985).
- (ix) Initiation of 50 - 100 L ethanol/day scale pilot plant studies (April 1985).

General Comments:

- (i) Serious effort should be made to ensure the presence of all the members of the Project Advisory Committee during the meeting. It is of paramount importance that the views and recommendations are heard collectively and discussed on a project of this magnitude.
- (ii) The Director of National Chemical Laboratory has to be complimented for his enthusiasm and his keeping track of up-to-date progress in the project in spite of his other commitments.

5.

Acknowledgments

It is my personal pleasure once again to thank Dr. Doraiswamy, the Director and all the investigators involved in the project for making the review process a memorable experience. I also wish to acknowledge the hospitality and friendliness of Drs. Maung and Runca during the de-briefing session in Vienna, Austria.

List of Publications

1. Continuous Ethanol production from sugarcane molasses by immobilized cells of an ethanol tolerant yeast
D.V. Gokhale, H. SivaRaman, S.G. Patil and C. SivaRaman
Presented at the VIIIth International Specialised Symposium on Yeasts, Bombay, 1983.
2. New and improved simple methodologies for evaluating controlled release larvicides
R.N. Sharma, N. Cardarelli, R.B. Mitra, V.B. Tungikar and P.D. Gund
Presented at the 10th International Symposium on Controlled Release of Bioactive Materials at San Francisco during July 24 - 27, 1983.
3. Enhanced production of extracellular B-glucosidase by Penicillium funiculosum in submerged culture
A.H. Lachke, K.B. Bastawde, V.K. Powar and M.C. Srinivasan
Biotechnol. Letters 5(10), 649 - 52, 1983.
4. Production and properties of B-xylosidase from Sclerotium rolfsii
A.H. Lachke, M.V. Deshpande and M.C. Srinivasan (Abstract)
Presented at the 52nd Annual Meeting of the Society of Biological Chemists of India, Poona, November, 1983.
5. Microbial Biomass Production by filamentous fungi grown on woody substrates
R. Rama Rao, S.S. Deshmukh and M.C. Srinivasan (Abstract)
Presented at the 52nd Annual Meeting of the Society of Biological Chemists of India, Poona, November, 1983.
6. Transfer and expression of DNA coding for cellulases from Cellulomonas uda to Bacillus subtilis
D.V. Gokhale, V.R. Srinivasan and D.N. Deobagkar (Abstract)
Presented at the XV International Congress of Genetics, New Delhi, Dec. 1983.

7. Isolation of a hypersecretive cellulolytic mutant (CU-1) of Penicillium funiculosum
M.C. Srinivasan, V.K. Powar, K.B. Bastawde and A.H. Lachke
(Abstract)
Presented at the VII International Biotechnology Symposium,
New Delhi, February, 1984.
8. Hydrolysis of cellulose by mycelial biomass of Penicillium funiculosum
M. Rao, V. Deshpande, R. Seeta, M.C. Srinivasan and
C. Mishra (Abstract)
Presented at the VII International Biotechnology Symposium
New Delhi, February, 1984.
9. Direct conversion of cellulose/hemicellulose to ethanol
Neurospora crassa
V. Deshpande, S. Keskar, C. Mishra and M. Rao
Presented at the VII International Biotechnology Symposium
New, Delhi, February, 1984..
10. Cellulase production by a mutant P. funiculosum UV-49
A.V. Joglekar, V.V. Jogdand, B.G. Gaikwad, P.M. Dalal
and N.G. Karanth
Presented at VII International Biotechnology Symposium
New Delhi, February, 1984.
11. Mass transfer effect in an immobilized yeast cell
packed bed reactor for ethanol production
N.G. Karanth
Presented at ICREC, Poona 9 - 11, 1984
12. Estimation of Ethanol in Fermentation Broth by solvent
extraction and gas chromatography
Varma, R., Swawant, U.D. and Karanth, N.G.
Enz. Microb. Technol. 6, 233 (1984).
13. Studies on contamination in Feed molasses in an
immobilized yeast cell reactor
R. Varma, P.M. Dalal, Chattopadhyay, S.K., B.A. Baliga
B.K. Ghosh, A.K. Srivastava and N.G. Karanth
J. Chem. Tech. and Biotechnol. 34B, 111 - 115 (1984)

14. Studies on cellulase production by a mutant Penicillium funiculosum UV-49
A.V. Joglekar and N.G. Karanth
Accepted for publication in *Biotechnology and Bioengineering*.
15. Occurrence of a procellulase in the culture filtrates of Penicillium janthinellum
V. Deshpande, S. Keskar, M. Rao and C. Mishra
Enzyme and Microbial Technology (In Press)
16. Production and properties of extracellular endoxylanase from Neurospora crassa
C. Mishra, S. Keskar and M. Rao
Applied and Environmental Microbiology (In Press).
17. Properties and applications of Penicillium funiculosum cellulase immobilized in a soluble polymer
M. Rao and C. Mishra
Biotechnology Letters 6, 5, 1984.
18. Continuous conversion of sweet sorghum juice to ethanol using immobilized yeast cells
U. Mohite and H. Sivaraman (1984)
Biotech. Bioeng. (In Press)

