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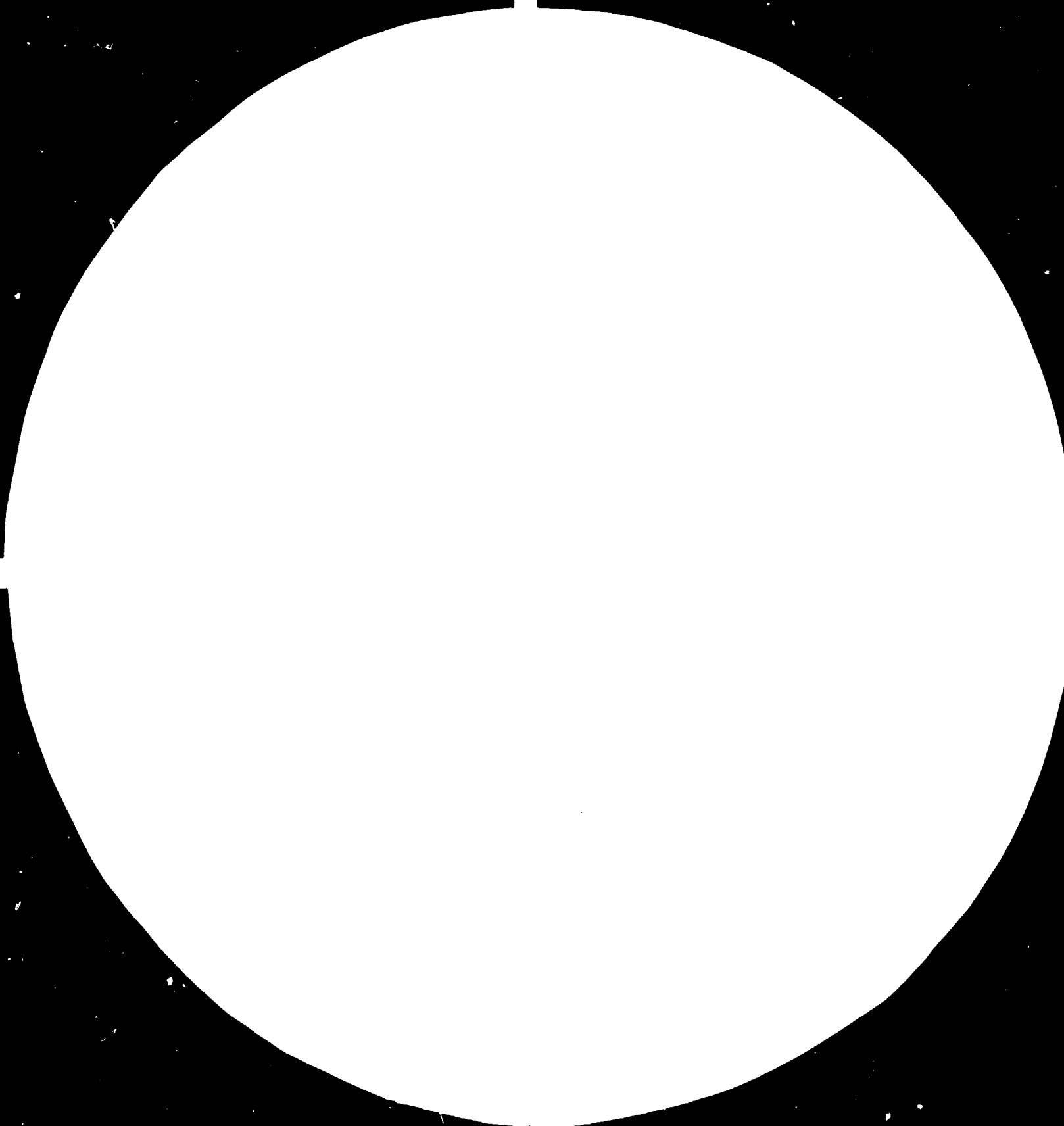

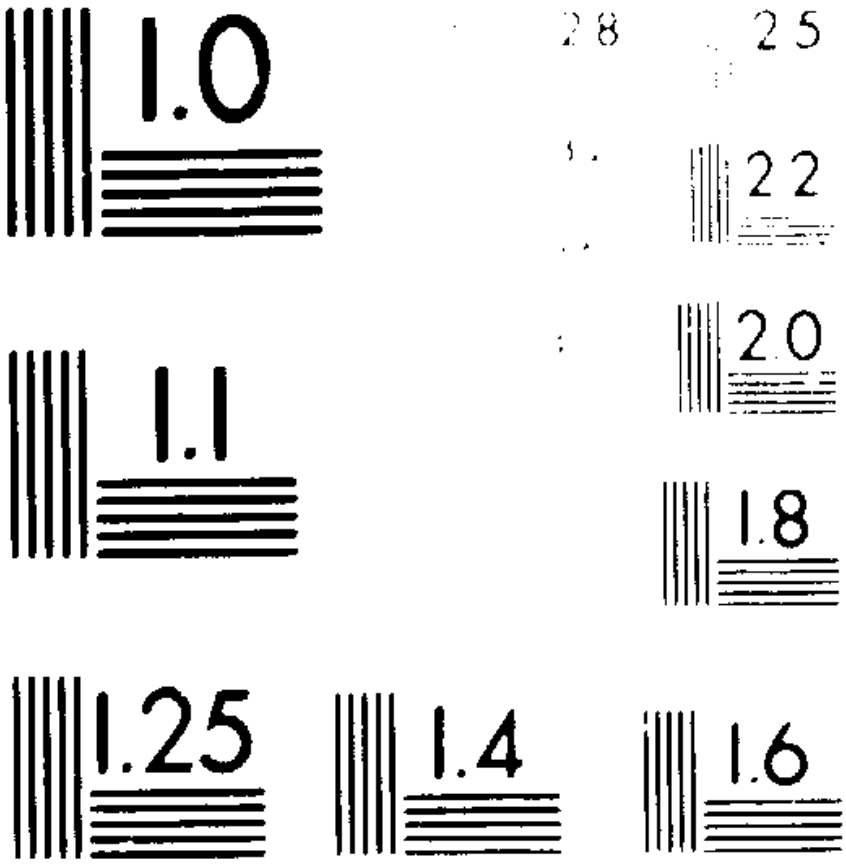

MICROCOPY RESOLUTION TEST CHART

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2 July 1983
English

India.

BIOSCIENCE AND ENGINEERING

DP/IND/80/003

INDIA

Technical report*

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

Based on the work of V.R. Srinivasan,
Chief Technical Adviser

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ABSTRACT

The project Bioscience and Engineering DP/IND/80/003 has been reviewed and recommendations for the immediate plan of investigations are set forth in this document. Of the immediate objectives as outlined in the project document, the ethanol process has advanced to such a state that one can foresee a tangible output by the end of the project tenure. In the area of enzymatic saccharification of cellulose, several interesting experiments have been carried out and from their results valuable information has been accumulated. It is suggested that the efforts in the microbial biomass program be better co-ordinated in order to standardize the methodology for growing microbes on a large scale. UN inputs to the program have been reasonably according to schedule except for certain difficulties in obtaining experts for consultation on time. Over-all out put of the project has been fairly satisfactory, which is reflected in the number of publications in international scientific journals, generated during the tenure of the project.

INTRODUCTION

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 immolbilized 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.

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.

Contributions: UNDP inputs $ 1,272,500

Government of India: Rs. 7,542,200.

Purpose of the Present Visit: The purpose of the present mission was to review the progress in the investigations on the biotechnologies of microbial biomass production from cellulose, enzymatic saccharification of cellulose to glucose and production of ethanol by immobilized cell reactors. Besides, suggestions are to be made to time target the experimental studies in order to accomplish the goals described in the project document.

Review of the present state of studies: Experimental studies carried out during the period of August 1982 to July 1983 were discussed extensively with individual investigators. A separate meeting was also held with the group leaders of the project in the presence of the area leader in order to discuss the program for the following year and to set target dates for the various experiments. The following summarizes the results of the studies with my comments:

I. Microbial Biomass Production:

(i) Studies of Penicillium janthinellum was carried out in fermentations for ascertaining optimal nutrient requirements with glucose as substrate. The doubling time was estimated to be 2.04 hrs.

(ii) Growth of Penicillium janthinellum was studied with rice straw as substrate. Different pre-treatment-methods such as alkali pre-treatment and steaming at differnt temperatures were used and the experiments were done in shake flasks. Alkali treatment was found to be superior to all other methods.

(iii) Several other sources of cellulose materials were tried as substrates for the organisms. All these experiments were in shake flasks.

(iv) Substitution of ammonium sulfate and peptone or yeast extract with cheaper and readily available complex nitrogen sources were attempted.

Comments: The experiments carried out are too diverse and open ended. Although these experiments generate some information, such experimentation does not provide valuable results which can be directly applied to advance towards the immediate objective of the project.

II. Enzymatic Saccharification of Cellulose:

(v) Penicillium funiculosum was mutagenized and two strains presumably producing increased cellulase activity were isolated.

(vi) Studies on the release of absorbed enzymes on the substrate were successfully completed enabling re-use of the enzyme.

(vii) Simple methods of concentration of dilute enzyme solutions were standardized.

(viii) Production of cellulose degrading enzymes in instrumented fermenter was studied in several experiments. However, the productivity obtained so far is far too low from the target.

(ix) Protoplast fusion as a method of transferring cellulase genes was investigated. Attempts to transfer the genes from Cellulomonas to Bacillus subtilis were successful and a few hybrid strains have been isolated.

(x) Rice straw pre-treated with ethylene diamine was used as a substrate for saccharification and it has been shown to be susceptible to enzymatic hydrolysis easily.

Comments: Progress in this area has been satisfactory. Based upon the results of these experiments, it should be possible to obtain enzymes with sufficient activity to saccharify cellulose to obtain increased concentration of glucose at a fairly rapid rate.

III. Conversion of Glucose/Cane Molasses to Ethanol:

(xi) Methodology for whole cell immobilization of yeast on gelatin has been standardized.

(xii) Studies on the growth of yeasts were carried out and the present knowledge is adequate for growing yeast in sufficient quantities required for the preparation of immobilized reactor with a capacity of 50-100 l/day production of alcohol.

(xiii) 10-20 l/day alcohol producing reactor has been fabricated and is in place.

(xiv) Studies on optimization of the reactor are in progress.

(xv) Initial studies on the pre-treatment of molasses for ethanol reactor have been completed.

Comments: This aspect of the program is the most straight forward and the majority of the targets set forth in programming during the last tripartite review have been attained. The progress has been so far satisfactory and the program is advancing as scheduled.

RECOMMENDATIONS

(i) Studies on the growth of Penicillium on different nitrogen sources may be postponed until the methodology for scaling up of the growth of the organism on standardized substrates is established.

(ii) It is recommended to set up experiments using one single cellulosic substrate with one pre-treatment process.

(iii) The preferred substrate may be rice straw and the method of pre-treatment be steaming to remove the hemicelluloses and delignification by ethylene diamine.

(iv) The choice of ethylene diamine as a pre-treatment agent was arrived at on the possibility of recovering approximately 80% of the ethylene diamine by solvent extraction. Furthermore as pointed out earlier, ethylene diamine pre-treated cellulose was found susceptible to enzymatic saccharification.

(v) However, since growth of the organisms on ethylene diamine treated cellulose has not been ascertained, growth on such a substrate may be compared to that on alkali pre-treated cellulose.

(vi) Growth parameters may be measured by determination of total dry weight and residual cellulose. Nitrogen determination of the end product my give false information if a small residual amount of ethylene diamine were present in the substrate. These experiments in shake flasks may be completed by the middle of September 1983.

(vii) Optimize the growth conditions to obtain maximal biomass production in 24 hrs. in instrumental fermenters through gradient feed of all the nutrients.

(viii) Initial experiments may be carried out using glucose as substrate at 0.2 and 0.4 and 1% level. Based upon the information obtained, subsequent studies may be designed with pre-treated rice straw as substrates.

(ix) The experiments referred to in vii and viii above may preferrably be completed during the period July 1983 to October 1983. [Refer to the published article from our laboratory. Production of single-cell protein from cellulose by Asp. terreus. Biotech. Bioeng. 25: 1509 (1983)]

(x) Studies on enzyme induction/isolation from cellulose grown mycelium may be completed till March 1984.

(xi) Methods for the isolation of absorbed enzymes and concentrations of dilute enzymes may be standardized in 1-5 l vessels.

(xii) Optimize biomass production in 100 l Chemap instrumented fermenter initially on glucose then on rice straw. From April 1984 onwards.

(xiii) Produce enzymes for saccharification in 100 l fermenter. April 1984 - .

(xiv) Studies on the growth of yeast to optimize the conditions for maximum productivity have to be continued.

(xv) Basic studies on tolerance of yeasts to high alcohol contact may be profitably pursued.

(xvi) The investigations on immobilized yeast reactor producing 20 l/day alcohol to be directed to obtain parameters for optimal designs. Present - December 1983.

(xvii) Based upon the result- of (xvi) preliminary design for 50-100 l/day alcohol production reactor is to be initiated till December 1983.

(xviii) Procurement of equipment and chemicals for the larger reactor to be completed by January 1984.

(xix) Construction of the reactor and auxilliary units are to be in place by July 1984.

(xx) Studies on the scaled-up reactor and process optimization may be carried out from July 1984.

GENERAL COMMENTS

(i) Better communication among the different individuals involved in the project coupled with more effective co-ordination may accelerate the program towards achieving immediate objectives.

(ii) The technology of utilization of ligno-cellulosics will be economically viable only if viewed as a systems concept, namely if the hemicelluloses and lignin are effectively used as by-products. In this context, it may be more attractive to develop the technology of utilization of cellulose to glucose as a single project with optimization of enzyme production as the main objective considering the biomass produced as a valuable by-product of protein for animal feed.

(iii) The over-all objective of the ethanol program is to obtain sufficient information to design a commercial plant producing 45,000 1/day alcohol. This will entail - at the last stage of the program - construction of a semicommercial plant producing 1000 1/day alcohol at an industrial site. The output of the project at NCL amy be a complete process design and operation at 100 1/day capacity. The program director and the scientists-in-charge are optimistic about the outcome of the project.

(iv) The second Tripartite Review of the project was held on June 30 and July 1, 1983. The status of the project, UN input such as study Tour, Training and equipment as well as the outputs of the project were discussed. A preliminary draft of the discussions was handed over to the substantive officer Mr. Maung during de-breifing.

Training

Discussed in Tripartite Review Report

Equipment

A complete list of publications generated during the tenure of the project is attached herewith.

ACKNOWLEDGEMENT

It is my pleasure to acknowledge the help rendered by all the investigators and the hospitality extended to me by the Director, Dr. Doraiswamy and Dr. C. SivaRaman, area leader, during my stay at NCL, Poona. The de-briefing session at Vienna, Austria was made enjoyable by the warmth and friendliness of Drs. M. Maung and Dagmar Runca.

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