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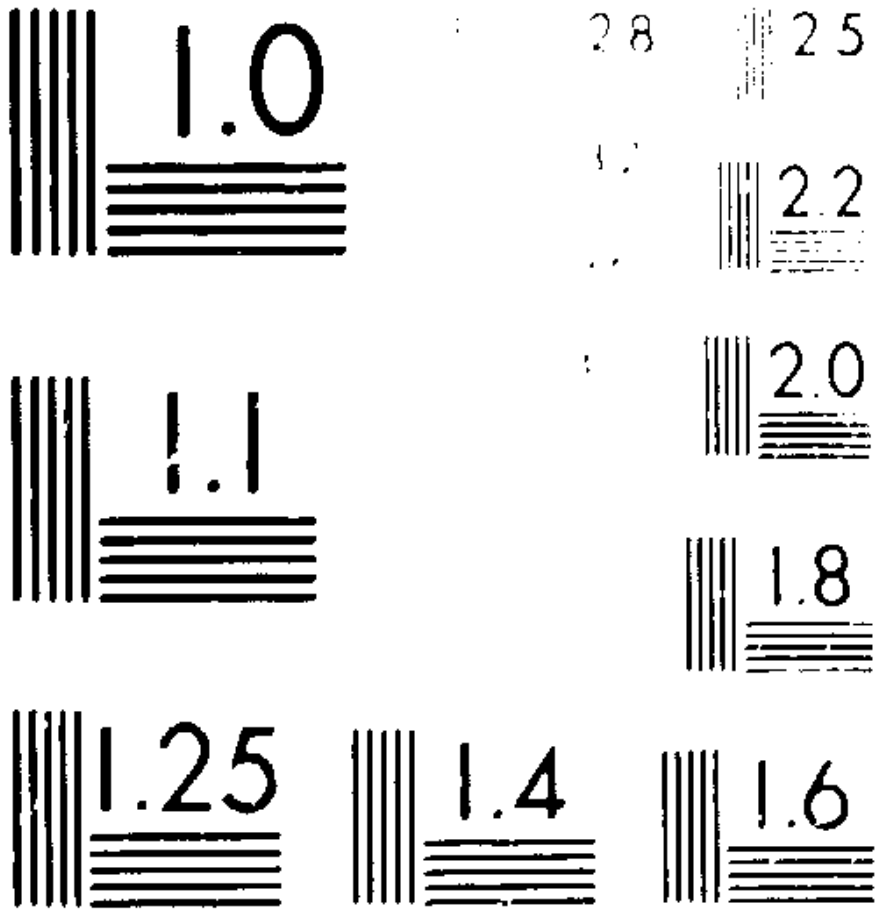
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# BIOSCIENCE AND ENGINEERING

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

United Nations Industrial Development Organization
Vienna

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## Table of Contents

## Abstract

Plant Biomass is a valuable renewable resource for the production of food, fuel and chemicals. The main objective of the project is to investigate the possibility and the development of methods of conversion of cellulose to protein and glucose as well as the production of ethanol by immobilized yeast cells. The objective of this mission is to review the state of the art in these investigations and to offer assistance in the mode of training young investigators to gain competence in the methodology of research and development in this valuable area.

## Introduction

Cellulose is one of the most abundant renewable resource found in the green plants. It is synthesized by the process of photosynthesis, along with hemicelluloses and lignin and form the bulk of the plant material. The annual production of cellulose is estimated to be approximately $10^{11}$ tons. Plant biomass is an extremely valuable resource for the production of food, fuel and energy as it is produced by direct photosynthesis from solar energy and the technology of utilization of this resource may play a vital role in the development of tropical countries.

Research on the utilization of cellulose for the production of microbial biomass product was initiated in National Chemical Laboratories, India about ten years ago. The project also included the investigations of saccharification of cellulose to glucose since glucose is generally used as feed stock for chemical and fermentation industries. These projects were supported by funds made available from the regular budget of the laboratory and from a Silver Jubilee Grant of the Council of Scientific and Industrial Research (India), New Delhi. Technical assistance for the project was also obtained from UNDP through the UN Food and Agricultural Organization during the period 1973-1978 (IND/73/018). Two consultants visited the laboratory for 2 man months and three scientists from the National Chemical Laboratory were trained for a total period of 18 man months in USA, UK and Sweden during the tenure of the technical assistance programme.

The present project was submitted as a project of Govt. of India for obtaining technical assistance from the United Nations Development Programme through the agency of UNIDO. It was approved by the UNIDO and implemented in September of 1981. The duration of the project is five years starting September 1981 - August 1986. Funds allocated by the UNDP is $ 1,272,500 and contributions by the Govt. of India amount to Rs. 7,542,200.

The objectives of the mission is to review the project and its original objectives and render assistance in the prosecution and accomplishment of its goals.

The main objectives of the project as outlined are as follows:

1. Development of a fermentation process for the production of microbial biomass product from cellulose.
2. Development of a process for enzymatic hydrolysis of cellulose to glucose.
3. Development of a process for the conversion of glucose to ethanol based upon immobilized microbial whole cells.
4. Development of processes for the production of controlled release pesticides by microencapsulation and monolithic matrix binding.
5. Establishment of NCL as a centre for Research and Development in the areas of biotechnology of cellulose utilization and controlled release pesticide formulations, providing services and disseminating scientific and technical information, training in research techniques in the relevant

areas, training abroad of NCL scientists in relevant areas of specialization.

The present report consists of an extended review of the first three objectives, a cursory review of objective 4 and the present status of the study tour of the senior scientists and training programme of the personnel as approved in the project. A more through review of controlled release of pesticides will be provided by the expert who is scheduled to visit in the month of October.

Present status of the project

Investigations carried out in the attainment of goals of different objectives were discussed with all the members of the groups. It was also emphasized that such group discussions among all the members of the group be encouraged to be held as often as possible so as to ensure cross-fertilization of ideas among the different investigators. The progress of the projects as a whole may be accelerated in such an interdisciplinary programme if there is a total commitment of the individual investigator to learn and contribute to the ideas and suggestions of members of different disciplines. The success of the project will be ensured if the groups interact as a single cohesive unit. The different groups presented their research conducted so far in their respective areas and their results are summarized as follows in this report:

1. Studies on Microbial Biomass Production

Pretreatment of rice straw for its bioconversion to protein by Penicillium janthinellum was investigated with a view to reduce the cost of pretreatment as well as to minimize the pollution problem due to alkaline effluents. Rice straw was soaked in water containing 0.5 - 1.0 g of NaOH per 10 g of straw, and steamed for 120 min. The suspension was then neutralized and used as such as carbon source for the growth of the organism. Such pretreated substrate gave yields of biomass with 20 - 22% protein.

Substitutions of reagent grade chemicals by commercial chemicals especially fertilizer grade ammonium sulfate as

nitrogen source did not result in significant diminution of the yields of biomass.

Semicontinuous production of biomass was attempted by the draw and fill method. About 80% of the culture of a batch fermentation was harvested after 48 h of fermentation keeping 20% for the next cycle. Fresh nutrient medium was replenished in the fermentor and the culture was allowed to grow for a further 48 h. The protein content of the biomass ranged from 13 - 17% in two successive runs and then started in decline.

Bagasse was subjected to pretreatment by exposing it to steam at 7 $kg/cm^2$ for 30 min and used as carbon source for P. janthinellum. Protein yields in such experiments were 5 - 7%.

Preliminary toxicity tests for MBP from P. janthinellum were carried out on mice by incorporating MBP upto 20% in the feeds for extended periods upto 12 months. No symptoms of toxicity were noticed and weight gain was comparable to the control fed on normal diet.

Enzymatic hydrolysis of cellulose to glucose

Using the wild strain of P. funiculosum, experiments were undertaken in instrumented fermentors to optimize the fermentation parameters in batch culture. Maximum F.P. activities (cellulase complex) of 3 IU/ml and productivities of 30 IU/ml have been obtained.

Work on mutation and strain improvement to achieve higher enzyme activity and productivity was continued. Several

mutants have been isolated by UV and chemical mutagenesis of P. funiculosum as well as Sclerotium rolfsii which give 30 - 50% higher FP, activities in shake flasks. A UV mutant giving 40 - 60% increase in FP activity in shake flask experiments when tried in an instrumented fermentor, confirmed the superiority in the production of FP activity.

Experiments were conducted on the methods of recovery of cellulase complex adsorbed on to the cellulose substrate in order to facilitate the re-use of the enzyme. Preliminary investigations on the addition of surfactants such as Tween-80 to elution buffers seem to enhance the recovery of the enzyme.

Conversion of glucose to ethanol by immobilized yeast cells

Experiments were carried out with yeast cells immobilized in an open-pre gelatin matrix using packed bed reactors for the continuous fermentation of sugar cane molasses to ethanol. The experiments were designed to study the effect of external diffusion on the rate of fermentation.

Some isolates of ethanol tolerant strains of yeast are being tested for stability under conditions of continuous production of ethanol. One of the isolates gave productivity of 55 g/l/h for 8% w/v ethanol concentration at conversion efficiency of 95% to 100% when used over a period of one month continuously.

Scale up studies are under way for the production of 10 to 20 litres ethanol/day. Conditions for the production of yeast cells under submerged conditions are being standardized using cane molasses medium with supplements. Equipment for the preparation of gelatin beads with entrapped yeast cells is being designed. Several pretreatment procedures for the clarification of molasses and conditions for prevention of

contamination of reactors are being examined.

Plan of future work

Production of yeast cells and immobilization for scale up studies will be standardized. The effect of parameters such as bead size, reactor design and configuration will be examined. Inexpensive pretreatment procedures adequate for suitably diluted molasses and prevention of the microbial contamination of the reactors would be studied. A pilot scale reactor with the production capacity of 10 to 20 litre ethanol/day will be set up. Preliminary cost benefit analysis of the pilot scale reactor is expected.

Preliminary studies on the membrane composition of ethanol tolerant yeast and efforts to improve ethanol tolerance through genetic manipulation will be initiated. The planned activity and estimated time for its completion is given schematically as shown in scheme (1).

Controlled Release Technology

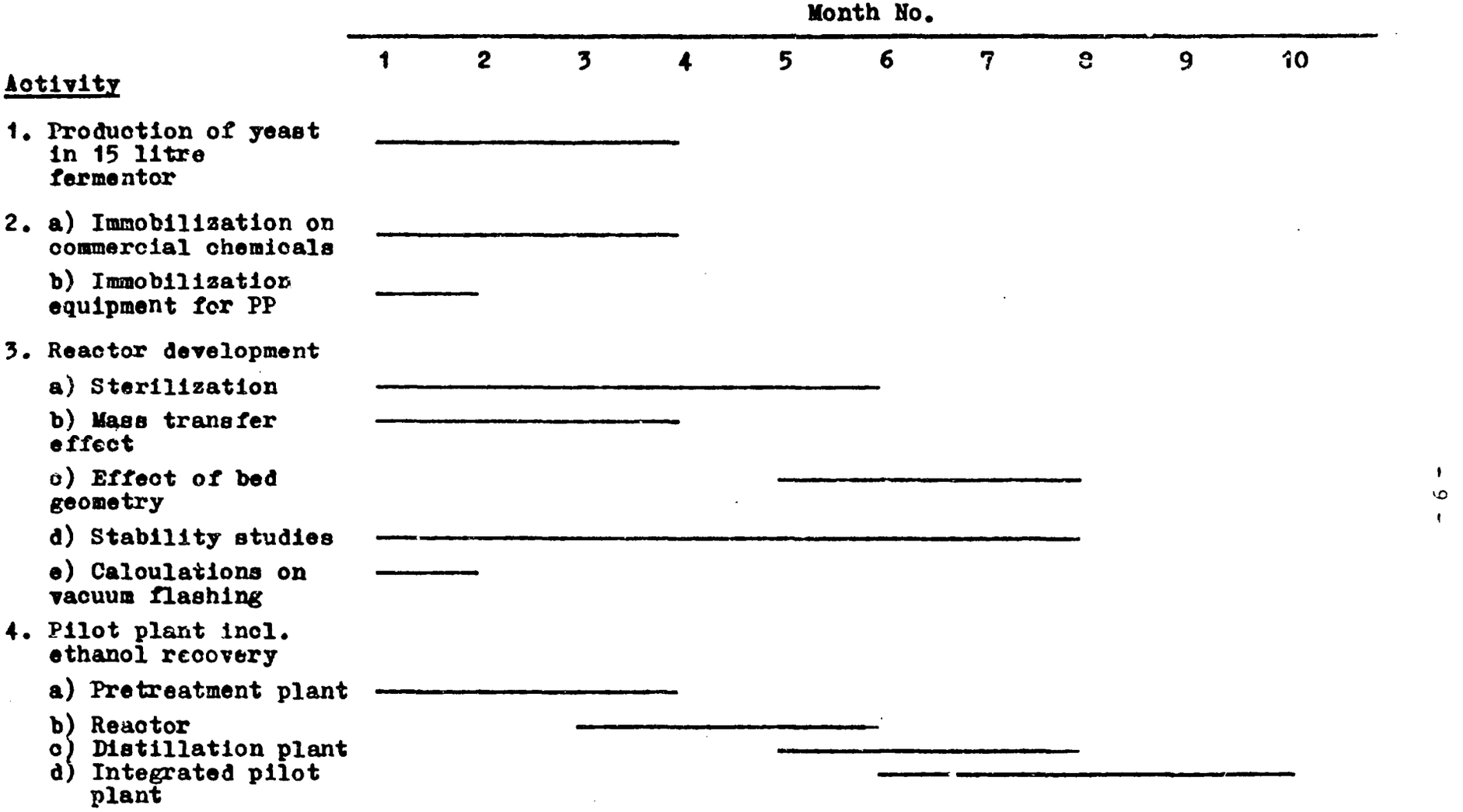
Satisfactory progress have been made in the encapsulation of carbofuran in starch xanthate and polyvinylalcohol matrices. The major problem encountered is the non-availability of carbofuran in pure form and sufficient quantities of abate and dursban.

Methods for determining the release rates for these formulations by chemical methods and bioassay are in progress.

Training

Study tour for 2 senior scientists as proposed in the original project document has been implemented.

Ethanol from molasses - immobilized cell process - bar chart starting month September 1982




Scheme (1)

Two scientists Drs. C. SivaRaman and N.G. Karanth have completed their visits to several laboratories during the period April 21 - June 10, 1982. I have examined their reports which are being transmitted to the agency.

The trainees have been selected and the schedule for their training has been prepared.

Equipment

The list of equipment obtained to date and those which have been requisitioned so far is submitted herewith in the Appendix.

There seems to be some delay (perhaps unavoidable) in obtaining equipment for the project. However, if the agency sends some information regarding the status of the purchase order, it may help the investigators to plan future investigations.

Recommendations

Most progress has been made only in the area of conversion of glucose to ethanol by immobilized yeast cells while the progress in the area of biomass from cellulose and cellulose to glucose has been rather slow. Hence it is recommended -

1. To prepare a conceptual scheme of the over all project as an integrated unit for the utilization of plant biomass as a renewable resource for the production of food, fuel and energy.
2. To identify the areas in the scheme as defined in the project document.
3. To streamline the investigations on the production of biomass and glucose as a single unit.
4. In order to accomplish recommendation (3) one single organism may be used for the production of biomass and conversion to glucose.
5. Optimize the environmental conditions for the growth of the organism on soluble substrates such as glucose at the initial stages.
6. Optimization may be preferably done in continuous cultures, for optimization under conditions of continuous cultivation, in my experience, facilitate the scaling-up of the process.
7. Enzyme production may be optimized as a batch process from the results obtained in (6) or as a continuous two stage process.
8. To interact more extensively with expertise available

in other groups at NCL for designing experiments on the improvements of strains used in the project.

9. Regarding trainees: During my discussions with Drs. SivaRaman and Mitra, it was pointed out that trainees in certain areas require more than six months and in other cases less than six months. Hence I recommend that under special circumstances, the training period may be made flexible as long as the cost of trainees in total does not exceed the allocated budget.

10. CTA The next visit of the Chief Technical Adviser may be scheduled in June 1983.

APPENDIX

List of Equipment

1981 Budget

| Item | Requisition No. | Actual Cost/ Est. Cost. US $ |
|---|---|---|
| I. Equipment Received | | |
| 1. Pharmacia Model P-3 Peristaltic pump with tubings. | 81/3 | 3,040.00 |
| 2. Shimadzu Model RIATF Gas Chromatograph with accessories. | 81/4 | 20,085.00 |
| 3. Hewlett-Packard Model 1082 B H.P.L.C. system with accessories. | 81/5 | 57,015.00 |
| 4. Shimadzu UV-visible Spectrophotometer Model UV-240 Graphicord. | 81/8 | 17,240.00 |
| 5. Toyota Crown 2 Model MS 112R SEKDS. | 81/13 | 8,285.00 |
| 6. Canon Model NP-400 Plain paper copier with accessories. | 81/16 | 10,870.00 |
| II. Equipment requisitioned on which no feedback has been received from UNIDO | | |
| 1. New Brunswick Model FS-314 3 Unit Laboratory fermentor 14 L capacity with Dissolved oxygen indicator recorder (3 No.) and auto pH controller/recorder Model pH 22 and accessories. | 81/12 | 25,000.00 |
| 2. New Brunswick Model DO-50 Dissolved oxygen indicator-recorder with accessories. | 81/1 | 2,830.00 |
| 3. New Brunswick Model pH 22 pH controller/recorder with accessories. | 81/2 | 4,845.00 |
| 4. Nitrogen determination system using Kjeldahl method. | 81/11 | 10,000.00 |
| 5. Infra-red gas analyser for $CO_2$. | 81/10 | 10,000.00 |
| 6. Paramagnetic gas oxygen analyzer. | 81/9 | 7,000.00 |
| 7. a) NBS model SF-116 standard Microgen fermentor 16 L capacity with accessories, piping and valve connections. | 81/14A | 41,000.00 |

| Item | Requisition No. | Actual Cost/ Est. Cost |
|---|---|---|
| b) Microprocessor control unit for above. | 81/14A | 41,000.00 |
| c) NBS-IF 150 Fermentor (100 L) with accessories and piping and valve connections. | 81/14A | 98,000.00 |
| 8. Beckman Model DU-8 UV-visible computing spectrophotometer with accessories and spares. | 81/15 | 30,000.00 |
| 9. Reciprocating water bath shaker New Brunswick Model R-86. | 81/6 | 3.285.00 |
| 10. Psychotherm Incubater shaker NBS Model G-27. | 81/7 | 10,000.00 |

1982 Budget

| Item | Requisition No. | Est. cost US $ |
|---|---|---|
| 1. Sorvall RC-5 Superspeed Refrigerated centrifuge with accessories. | 82/1 | 15,000.00 |
| 2. LKB UV Monitoring system with accessories | 82/2 | 15,000.00 |
| 3. Beckman Paramagnetic oxygen analyzer | 82/3 | 8,000.00 |
| 4. Modular type universal mill with accessories. | 82/4 | 18,000.00 |
| 5. Buchi Model RB-120 Rotavap with accessories. | 82/5 | 1,500.00 |
| 6. Shimadzu top loading digital balance | 82/6 | 1,200.00 |
| 7. Shimadzu UV-visible recording spectrophotometer Model UV-240 | 82/7 | 18,000.00 |
| 8. NBS Model DO-50 dissolved oxygen indicator/recorder with accessories. | 82/8 | 6,000.00 |
| 9. LKB multi perpex peristaltic pumps. | 82/9 | 4,000.00 |
| 10. Glucose analyzer YSI Model 23A-230 with accessories. | 82/10 | 6,000.00 |
| 11. Essential accessories for HPLC Howlett-Packard Model 1082 B. | 82/11 (Old 81/5 continued) | 15,000.00 |
| 12. Analytical balance mettler (semi-micro). | 82/12 | 1,000.00 |
| 13. Mini spray drier Buchi Model 190. | 82/13 | 11,000.00 |
| 14. Beckman Infra-red $CO_2$ analyzer. | 82/14 | 10,000.00 |
| 15. Psychotherm Incubator shaker NBS Model G-27 with accessories. | 82/15 | 10,000.00 |
| 16. pH controller Gallenkamp Model FBL-720. | 82/16 | 5,000.00 |