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BIOSCIENCE AND ENGINEERING

DP/IND/80/003

INDIA

Technical Report *

Mission 15 November to 3 December 1985

Prepared for the Government of the Republic of India
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ABSTRACT

Studies carried out under the project Bioscience and Engineering DP/IND/80/003 during the period from August, 1984 to November, 1985 were reviewed. General comments of the overall progress and recommendations for further research and development to accomplish the major objectives outlined in the original proposal are reported here.

Investigations on Cellulose Biotechnology concentrated upon the evaluation of mutants of Penicillium funiculosum for enzyme production and the environmental conditions for increased enzyme synthesis. Progress in this area has been slow and more intensive and novel experimentation is required to approach the goals. In the area of immobilized yeast for ethanol production satisfactory progress has been made in identifying some of the possible causes for the breakdown of the biocatalyst after a short-term operation and laboratory experiments are being continued to overcome the difficulty of long-term operation of the reactor. The investigators are still optimistic about the successful outcome of the project in bringing the studies to a pilot and demonstration phase.

Project Background: The project DP/IND/80/003 was requested by the governement of india with the overall objective to strengthen the expertise and research facilities available at the National Chemical Laboratories (NCL) in biotechnology or 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 th. 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.

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.

Purpose of the Present Mission:

(i) Review the progress and advise on further research in the area of a) optimization of production of biomass and cellulases and enzymatic saccharification of ce-lulose and b) production of ethanol by immobilized cell reactor.

(ii) Participate in a tripartite (NCL, CSIR and UNDP) review of the project.

Due to certain exigencies, the tripartite review was postponed. However, the project status report for the review was prepared by the project leaders in the National Chemical Laboratory and a copy of which was handed over to Dr. Maung, officer-in-charge of the project at UNIDO in Vienna.

Review of the State of Studies: Previous reports have outlined the procedures followed for reviewing the project. Following the same procedure, the work carried out during the period of August, 1984 to November, 1985 was discussed with the individuals who actually performed the experiments. The results were discussed as two separate groups (one dealing with cellulose biotechnology and another with the production of ethanol by immobilized cells). Further, two separate meetings were held with the group leaders of the project with the Director of National Chemical Laboratories present in both the discussions.

Besides, during this visit, actual experimentation was done in scaling up of growth of Penicillium funiculosum from laboratory 10 l fermenters to the highly instrumented Chemap fermenter of 100 l capacity. Although the experiment was not completely successful it gave an insight to further studies required to make a successful run.

The outcome of the review process is outlined as follows:

a) Cellulose Biotechnology:

(i) Three different mutants of P. funiculosum were isolated and evaluated for enzyme production in shake flasks. Dr. M.C. Srinivasan discussed a unique method of recognition of mutants having a higher potential for enzyme synthesis in the presence of Cu^{++}.

The mutants showed twice the cellulolytic activities of the wild type: however, they had only 30% of the activity of Trichoderma reesii C-30 strain.

(ii) Cellulose was pelletized and added at intervals during the fermentation of Penicillium funiculosum in laboratory fermenters in order to increase the total amcunt of enzyme produced. The activities were found to be maximum after 9 days of fermentation and thereafter a decrease was noticed.

(iii) Hydrolysis of 2 kg of alkali-treated bagasse was carried out in a stirred tank reactor using appropriate concentrations of enzyme from P. funiculosum (10 I.U./g of substrate). A 5-5.5% stream of glucose was obtained with 73% of cellulose saccharified in 24 hrs. However, when the experiment was repeated with all the enzyme present initially and bagasse was added at intervals the result was 6% glucose at 10 hrs with 60% utilization of cellulose.

b) Immobilized Cell Technology for the Production of Ethanol: The bioreactor with S. uvarum in Ca-alginate beads operated with 10 liter alcohol/day production only for 10 days. Then the reactor deteriorated resulting in low alcohol production. Molasses was used as the raw material.

Studies were scaled down to laboratory experiments. The column was operated successfully for the target period of 3 months at the desired conversion efficiency of 95% with a productivity of approximately 15 g l^{-1} h^{-1} at 6.5% ethanol, using sucrose as the raw material.

In order to find the effect of high salts on the life of the bioreactor, an equivalent concentration of salts present in molasses was used along with sucrose. The experiment was in progress. The efficiency of the reactor remained unaltered even after two weeks.

If the deterioration of the bioreactor were due to the loss of viability of cells in the presence of high salts concentration, short-term pulsing with nutrients may help to increase the cell densities and restore the efficiency of the reactor. Such an experiment was performed: however, the results were inconclusive.

General Comments and Recommendations:

1) The investigators in the area of ethanol production by immobilized cell technology have been working as a cohesive unit and the work is progressing satisfactorily. However, it seems that more co-ordinated intensive effort is needed in cellulose biotechnology to obtain tangible and useful information.

Studies on the growth of Penicillium are stymied probably due to lack of available information on the physiology of growth of fungi in general. It is my experience that differentiating organisms during the phase of the rapid vegetative growth do not use biochemical pathways requiring oxygen and are highly sensitive to excess oxygen.

Therefore, my recommendations are:

(i) Using gradient-feed technique which gives a fairly physiologically synchronous state of rapidly growing mycelium, effect of O_2 tension may be determined on the product yield at low, medium or high

dissolved oxygen concentration. These experiments may be run for not more than 24 or 48 hours in instrumented fermenters. If the experiments are inconclusive, it is worthwhile to run the experiments in the pilot fermenter.

2) After determining the proper aeration the experiments may be conducted in the Chemap fermenter with 1, 2 and 4% pre-treated cellulose as substrates. The experiments should not be more than 24-48 hrs duration.

3) Based upon the experiments cost analysis of a reasonable size plant for the production of Microbial Biomass may be performed. The analysis preferably include a breakdown of procurement cost, pre-treatment cost, fermentation as well as down-stream processing costs. This type of analysis will provide information to which step in the process development requires further investigation to make the cellulose technology a viable industry.

4) Similar experiments have to be carried out to determine the effect of O_2 tension and pH for increased enzyme production. The detailed experimental protocol has been discussed with the group of investigators.

It may be worthwhile to have a cost estimate for the production of enzymes for cellulose degradation. Even if the saccharification of cellulose may not be economical, there may still be a process for industrial enzyme manufacturers.

After discussing the breakdown of the bioreactor for ethanol production and the experiments done so far I am of the opinion that the salts present in molasses may not be primarily the cause of breakdown, although my opinion is not shared by some of the investigators. It seems to me that the waxy material in molasses may adhere and coat the Ca-alginate beads thereby causing diffusion limitation for the substrate. This problem may be overcome by a different configuration of reactor design and operating conditions as discussed by

Mr. Pendse and Nene, the bio-engineers of the group. The following are my recommendations.

A. Clarification of Molasses: i) Molasses may be clarified at pH 2.0 instead of the higher pH used a present. ii) Clarification may be attempted by filtration through activated carbon sand or other inexpensive filter aids.

B. Laboratory Reactor Operation: i) Operate a laboratory reactor using clarified desalted molasses. ii) Operate the type of reactor discussed above with molasses as used at present.

C. Yeast Growth Adaptations: i) The yeast required for immobilization for the newly designed reactor, may be advantageously grown in Chemap 150 l fermenter which will yield a uniform quality product. ii) Use continuous culture as a technique for selection of yeast tolerant to high salt and/or ethanol.

Acknowledgement:

It is my pleasure to thank Dr. Doraiswamy and the other investigators for their patience and forbearance during my visit and to extend my appreciation for their generous hospitality. I would like to acknowledge the friendliness and hospitality of Dr. Maung and the UNIDO staffduring my de-briefing in Vienna, Austria.