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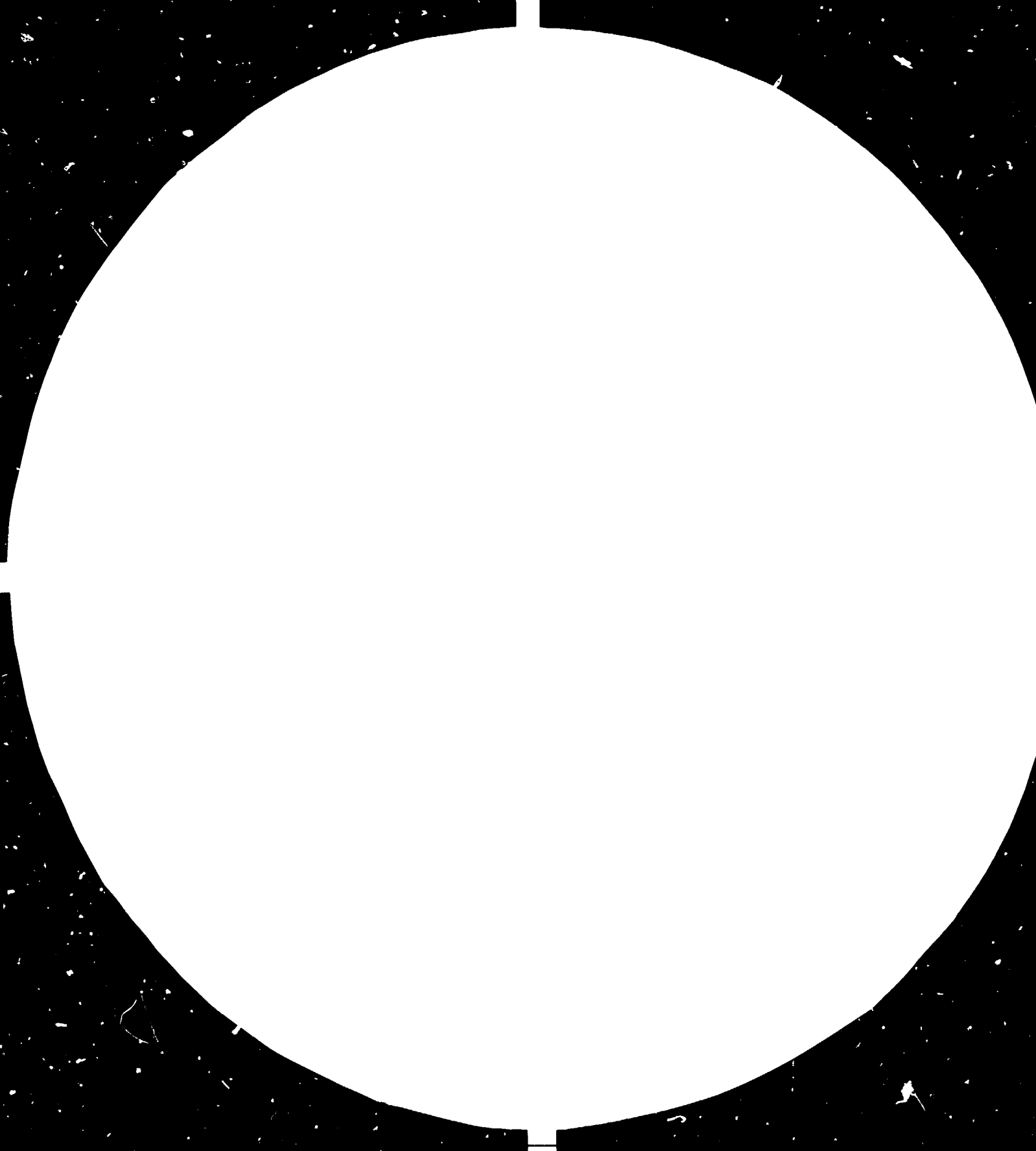
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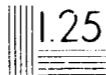
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ABSTRACT

A two-week visit to the National Chemical Laboratory, Pune, India included participation in the International Chemical Reaction Engineering Conference and detailed discussions of the UNIDO project "Bioscience and Engineering" (IND/80/003). The aim was to strengthen the expertise and research facilities for the exploitation of ligno-cellulose resources and of technologies for controlled release pesticide formulation. This report contains comments and advice on :

- production of microbial biomass from ligno-cellulosic materials through direct microbial conversion techniques;
- strategies for the improvement of protein yields;
- reactor design developments.

1. Introduction

The National Chemical Laboratory (NCL) is a multidisciplinary research institute which, among others, is carrying out research and development in biochemistry, microbiology and biochemical engineering. Under the UNDP/UNIDO project DP/IND/80/003, Bioscience and Engineering, the following activities are presently pursued :

- development of a fermentation process for the production of Microbial Biomass Products from cellulosic materials;
- development of an enzymatic process for hydrolysis of Cellulose to Glucose;
- development of a process for Glucose conversion to Ethanol based on immobilized microbial whole cells;
- techniques for pesticides immobilization for controlled release of pesticides involving microencapsulation and monolithic matrix.

The visit started with participation in the International Chemical Reaction Engineering Conference. There were extensive discussions with Indian and other delegates to the meeting, and the following paper was presented :

H.R. Bungay "Population Control in Continuous Fermentation" in Frontiers in Chemical Reaction Engineering, ed. L.K. Doraiswamy and R.A. Mashelkar Vol. 1, Wiley Eastern 1984, pp. 573-579.

This paper is relevant to the UNIDO project in that a technique is described for overcoming contamination or for maintaining two different strains for a synergistic fermentation.

A portion of the review of the UNIDO project coincided with the reviews of two other consultants, Dr. J. Bu'Lock and M. Moo-Young. The first section of this report covers my interpretation of the views of the consultants working jointly and the second section is my independent recommendations.

2. Joint Recommendations of Consultants Messrs. Bu'Lock, Moo-Young and Bungay

Bu'Lock, Moo-Young, and Bungay recommend that optimization of P. funiculosum wild-type be continued but at a lowered priority. Some large scale runs should be performed to obtain enzyme for research and development of the saccharification step. Furthermore, glucose from saccharification should be substituted for the pure glucose presently being used for optimization of the growth of P. funiculosum.

We very strongly recommend that high priority be assigned to mutation and screening to find hyperproducing strains of P. funiculosum. The wild type is a better producer of cellulases than are the wild-type strain of other investigators, so the probability of developing outstanding mutant cultures is high. A high-yielding cellulase fermentation using mutants from NCL would be marketed in

its own right. In any event, the cost of cellulases is a crucial factor in converting biomass to ethanol, and a major improvement in this fermentation is desperately needed.

Bu'Lock and Bungay sense the need for flocculating strains of yeast in case the NCL immobilized yeast process is uneconomic. Moo-Young recommends instead the use of yeast attracted to wood chips. These are both interesting alternatives to immobilized yeast and deserve attention at NCL but only at a second level of priority.

Bu'Lock and Bungay also emphasize the importance of organisms that can simultaneously hydrolyze cellulose and produce ethanol or other products. This may also be conducted by mixed cultures of microorganisms. In any event, it is likely that anaerobic species such as Clostridia will be involved. It is recommended that NCL assign a high but not urgent priority to testing of organisms with potential for direct fermentation of cellulose.

Bu'Lock, Moo-Young and Bungay agree that pilot plant testing of the fermentation of molasses to ethanol by immobilized yeast is worthwhile. There may be valuable spin offs such as the demonstration of heat pump technology. However, this project should have definite time limits and should not strive for a very fine degree of optimization.

Moo-Young and Bungay recommend that rough, preliminary cost estimates and a provisional plant design be performed for a biomass to ethanol factory using NCL technology.



Whenever possible, data from NCL should be used. Should there be gaps for which no data are available, NCL scientists and engineers should furnish their best guesses or opinions to the cost analysis group. Such a cost exercise should have great value in highlighting the areas of uncertainty and in assessing the importance of various projects.

Whenever there is a significant advance in the laboratory or pilot plant, the cost exercise should be repeated.

An example of the importance of cost analysis comes from the Iotech Corporation. Their analysis showed a serious problem because of the temperature of the fermentation that produces the cellulase enzymes. In North America, the temperature of available cooling water is relatively low. Nevertheless, the temperature difference between the cooling water and the fermentation meant that a rather large amount of cooling area would be required, and the necessary cooling coils would be quite expensive. The scientists who are developing strains for the Iotech process are now selecting organisms that function at higher temperatures, and there will be an appreciable reduction in the cost of cooling coils for the large-scale fermentation.

At present, the NCL enzyme fermentation operates at 30°. It is likely that a cost analysis will show that cooling water in India is inappropriate for cooling this fermentation. If so, the NCL scientists are well aware of methods for selecting microorganisms that function at higher temperature.

3. Recommendations made by Mr. Bungay

Bungay recommends that scale up of the saccharification step be scrutinized. This step with abundant sugar and enzymatic protein provides a rich medium for the growth of contaminating organisms. The contamination problem was never solved at Natick Laboratories, and they resorted to a temporary expedient of adding mercurial germicides. The Iotech group hopes to keep this steam-exploded cellulosic material sterile, but they are very worried about contamination at the saccharification step. The NCL material will not be sterile, and the cost inputs of special precautions or of sterilization prior to saccharification should be assessed.

Bungay also recommends that recycle of spent beer after ethanol distillation be considered. A portion might be recycled either to the enzyme fermentation or to the ethanol fermentation. The ethanol fermentation of corn grain is now being operated in the U.S. with a very high degree of recycle to save on make up water and nutrients and to greatly reduce the volume of spent beer sent for waste treatment. Although the NCL project using sugar cane molasses has a greatly reduced opportunity for recycle because of high salt content, any reuse of even a small fraction of spent beer will be advantageous. When the switch to glucose from the hydrolysis of cellulose is made, the chance for successful recycle becomes quite high.

A suggestion rather than a recommendation is that chemical reactions of the sugars from hemicellulose be considered. The formation of furfural is reported to be easy and the methods are given in considerable detail in the literature. There also is interest in some laboratories in making derivatives of the cellulose from biomass refining.

4. Other activities

Computer interfacing, computer simulation, and teaching exercises were discussed. Several programs used routinely at Rensselaer Polytechnic Institute (RPI) were successfully operated on the NCL microcomputers.

With sufficient time for detailed interactions, many projects and ideas not in the main stream of the UNIDO project were reviewed. Both the consultant and NCL benefited. Lamellar sedimentation for the collection of yeast cells has been demonstrated at R.P.I. and may have application at NCL.

5. Conclusion

The arrangements and hospitality of the NCL group were excellent. The Director, Dr. Doraiswamy, has a strong interest in biotechnology and is highly supportive of the UNIDO project. The detailed research and development is in very good hands, and Drs. Srinivasan and Karanth are especially commended on their excellent and innovative approaches.

This project is an ambitious and difficult undertaking. Progress has been impressive, and the chances for success are reasonably good.



**84.08.22**