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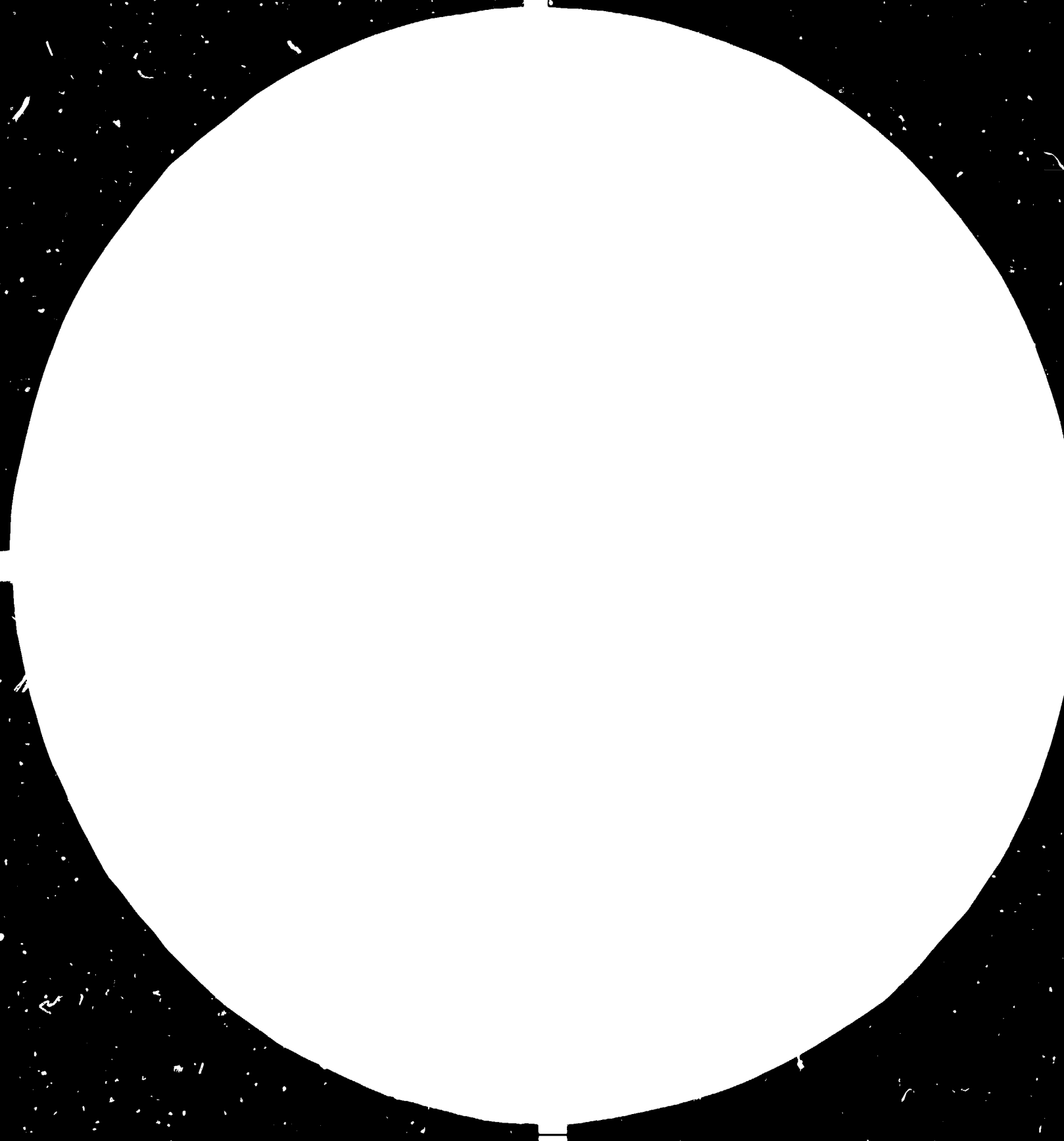


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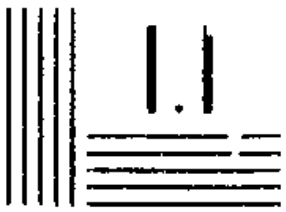
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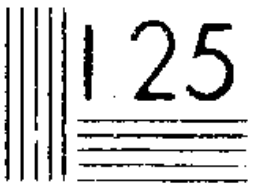
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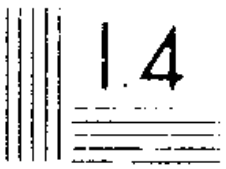
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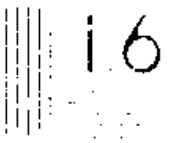
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RESTRICTED

June 1984

ENGLISH

BIOSCIENCE AND ENGINEERING

DP/IND/80/003

INDIA

Technical Report*

Mission 7-22 January 1984

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

Based on the work of Murray Moo-Young,
Consultant on Biotechnology
Conversion of Cellulose to Microbial Biomass Product

United Nations Industrial Development Organization
Vienna

* This document has been reproduced without formal editing.

1. Background

The terms of reference of this UNIDO mission called for a consultant's advisory assistance to the National Chemical Laboratory (NCL) at Pune in its biotechnology fermentation programs, especially those dealing with bioconversions, cellulose-to-microbial biomass (in particular), cellulose-to-glucose and glucose-to-ethanol. The mission covered a 16-day period in Pune and associated in-transit rest-stops (January 7-22, 1984) with no briefing or de-briefing stopover in Vienna.

The lack of material mailed from Pune to Waterloo are the main causes for delay of submission of this report. In fact, at the time of writing, the material has still not arrived and aspects of the report rely heavily on memory.

As part of the mission, the first three days (January 9-11) were spent at the NCL-organized "International Chemical Reaction Engineering Conference"(ICREC). An outline of the ICREC is given in Appendix A. ICREC afforded a unique opportunity to discuss and exchange information with some of the world's foremost authorities on chemical reactors: biochemical reactor design and operation were of direct relevance to the mission objectives. The remaining nine days were mainly spent in NCL laboratory visits and meetings with the two key research leaders and their staff who are involved with the project: Dr. M.C. Srinivasan of the Biochemistry Division and Dr. N.G. Karanth of the Biochemical Engineering Group (within the Chemical Engineering Division) at

the NCL. An NCL-prepared outline of the structure and research activities, including a list of facilities and publications of the Biochemical Engineering Group, is given in Appendix B. A similar outline for Biochemistry was not given to the writer; however, the relevant research activities, publications and extent of collaboration with the Biochemical Engineering Group are summarized in the July 3, 1983 Technical Report of Dr. V.R. Srinivasan, the Chief Technical Advisor to this project.

In addition to the individual and group meetings at NCL referred to above, the following seminars, lectures and external visits were made:

(a) ICREC lecture on "Biorectors for solid-substrate aerobic fermentation: design options for the Waterloo SCP process"; (b) Visit to the Pudumjee Pulp and Paper Mills, Pune; (c) Visit to the Tata Research Development and Design Centre, Pune; (d) Seminar on "Process biotechnology" at the Hindustan Antibiotics Company, Pune; (e) Seminar on "Bioreactor design" at the Indian Institute of Technology, Bombay.

It should be pointed out that during the Pune visit, several meetings were also held with Dr. John Bu'Lock, UK, Dr. K. Schügerl, FRG, and Dr. Henry Bungay, III, USA, who are also consultants to this project and were in Pune during this visit. In fact, most of the following findings and recommendations were derived from collective deliberations. For convenience, these results are itemized below moving from generalities to

specifics and in no particular order of importance. Strictly interpreted, some of the items fall outside the scope of this mission but are considered to be sufficiently related or interesting to be included.

2. Findings and Recommendations

2.1. The NCL has made a major commitment to biotechnology research, in general, and fermentation technology, in particular. Within this UNIDO project, the latter aims at the utilization of lignocellulosic materials for the production of edible protein-rich microbial biomass, glucose feedstock and fuel-grade ethanol. As such, the NCL is in competition with many other research groups throughout the world which hope to demonstrate that, by bioconversion processes, lignocellulosic biomass can be used as a "renewable resource". Like many of the other groups, the NCL does not seem to have made comparative locally-relevant assessments of the economics of alternative raw materials such as starch and molasses, which are more easily utilized in the proposed processes, or of the commercial viability of alternative product types such as soybean meal, oil-cakes, methane, methanol, furfural, etc. produced by other biochemical and/or physicochemical processes. In the various evaluations, the possibility of by-product credits also need to be addressed, e.g., lignin from pretreatment, CO_2 from EtOH production.

2.2. The research capability of the scientists and engineers involved in the fermentation program is of world-class calibre, as reflected in the quality and quantity of papers they have published in reputable

journals (see Appendices C and D). However, there is need for analyses of these research results to identify possible "bottlenecks" in a process and to examine their possible commercial usefulness for the Indian marketplace or for their technology export value. In addition, contacts with potential industrial users of the various process developments should be established.

2.3. It is recognized that native lignocellulosic materials are biochemically recalcitrant and some pretreatment is required before further processing. For a proteinaceous microbial biomass product (MBP), the NCL team has found a cellulolytic fungal organism, Penicillium janthinellum, which grows well on rice straw after alkali pretreatment. In addition, other cellulolytic organisms, Penicillium funiculosum and Neurospora crassa, are found to be good producers of extracellular cellulase enzymes (see later) and it is proposed that the residual MBP from these enzyme-production processes may be suitable as animal feed. Two points should be made on these issues: (a) Good extracellular cellulase enzyme-producers are not likely to be good MBP-producers since, bioenergetically, these are based on opposing biosynthetic processes, (b) Preliminary in-vivo animal feeding trials should be carried out as soon as an MBP process shows promising signs of being economically viable (in terms of both growth rate and yield) since product quality could become over-ridingly important.

2.4. While there may be some merit to NCL's screening program to try to find their "own" MBP organism isolates, they may be "re-inventing the

wheel" in the sense that the Waterloo SCP process development (see Appendix E). has already carried out such a screening. In any case, this process could be used as a base reference for additional screening by NCL. One member of the NCL research team, Mr. V. Jogdand, is now scheduled to undergo a 6-month training program on this and related biomass conversion processes at Waterloo, starting in the Spring of 1984. In addition, industrial interest on the part of a local company, Pudumjee Pulp and Paper Mills, should be followed up by NCL with the writer offering to act as a coordinator.

2.5. Similarly, the search for other organisms with cellulase enzyme-producing capability should focus on optimizing the application of the current C30 mutant which already appears to compare favourably with other publicly-available strains. The relatively good cellobiase-component activity compared to the world reference, T. reseii, should be exploited in the glucose-from-cellulose process strategy. A fed-batch culturing approach appears to be working well and more research at optimization is encouraged. Cellulase is also being examined as a product for de-skinning, e.g. tomatoes.

2.6. As a prerequisite to point 5 above, there is an urgent need to examine the relative economics of various pretreatment techniques for the lignocellulosic raw materials, e.g. via steam explosion, solvent extraction, acid hydrolysis, with or without catalysts. These techniques have already been extensively researched elsewhere and only their economic implications here need be considered.

It is the feeling of the author that it would be very uneconomical to make glucose in commercial quantities from native cellulosic materials in India at the present time.

2.7. For the production of ethanol from sugar solutions, the NCL team is to be commended for having developed a gel-entrapped immobilized-yeast packed-bed bioreactor system which has been demonstrated at a pilot scale using a column, ≈9" diameter and ≈2' high. It is claimed that alcohol productivities of 60 g/L•h at 90% molasses-base conversions over 6 to 8-week periods have been obtained, these performances being comparable to other research claims elsewhere. However, it should be noted that other known techniques using surface-attachment of cells to inexpensive packing materials such as wood chips as done in the writer's laboratory (see Appendix F) may be more economical than the NCL gel preparation and entrapment strategy; in addition, the possibility of intraparticle diffusion limitations is avoided. The visiting scientist, V. Jogdand, will also study this technique during his Waterloo visit.

3. Concluding Remarks

The present NCL team expertise has the essential multidisciplinary characteristics to carry out the envisaged project activities. The existing and expected research facilities appear to be adequate for achieving the immediate exploratory goals. The writer would rate as "satisfactory-to-excellent" the present status of all the three inter-related projects,

i.e., microbial biomass production, cellulose-to-glucose conversion, glucose-to-ethanol conversion. However, if other recommended process options are to be examined, further funding may be required. In addition, sensitivity analyses of the technoeconomic feasibility of the various processes for Indian relevance should be initiated as soon as possible. In the latter two aspects, this writer would be happy to contribute to the exercises.

The Indian hosts are to be complimented on having arranged well-organized, co-ordinated, useful meetings. In particular, the opportunity of having concurrent discussions with other consultants involved with the project (Bungay, Bu'Lock, Schügerl) allowed debates leading to certain degree of consensus. Finally, mention should be made on the warm hospitality received from the hosts and the Indian people, which allowed efficient and enjoyable work.

D. Runca/as

UNITED NATIONS

APPENDIX A

14 June 1983

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

UNIDO

JOB DESCRIPTION

DP/IND/80/003/11-56/32.1.C

Post title	Consultant on Biotechnology: Conversion of Cellulose to Microbial Biomass Product (MBP)
Duration	2 weeks
Date required	8-20 January 1984
Duty station	Pune, India
Purpose of project	The aim of the project is to strengthen the expertise and research facilities at the National Chemical Laboratory (NCL) for the development of biotechnologies for exploitation of ligno-cellulosic resources and of technologies for controlled release pesticide formulation. More specifically the project is aimed at development of fermentation process for the production of Microbial Biomass Product (MBP)from cellulose; development of enzymic process for hydrolysis of Cellulose to Glucose (CTG); development of a process for conversion of Glucose to Ethanol based on immobilized microbial whole cells (GTE).
Duties	The consultant will assist in an advisory capacity the scientists from the National Chemical Laboratory in their fermentation programme and is required specifically to advise on: - production of microbial biomass from ligno-cellulosic materials through direct microbial conversion techniques; - strategies for the improvement of protein yields; - reactor design developments. He will be expected to attend the International Chemical Reaction Engineering Conference 9-11 January 1984, Punce, organized by the NCL. He will submit a brief report on his findings and recommendations.

..../..

Applications and communications regarding this Job Description should be sent to:

Project Personnel Recruitment Section, Industrial Operations Division

UNIDO, VIENNA INTERNATIONAL CENTRE, P.O. Box 300, Vienna, Austria

Qualification:	High level scientist/Engineer with vast experience in research and application of microbial technologies for conversion of ligno-cellulosic materials into proteins.
Language	English
Background Information	The National Chemical Laboratory is a multidisciplinary research institute which, among others, is carrying out R+D work 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.

PROGRAMME

The programme will include plenary lectures, contributed lectures and a panel discussion. The contributed lectures will be run in parallel sessions classified as per the following theme:

- Biochemical Reactors (BR)
- Dynamics and Stability (DYN)
- Fluid Bed Reactors (FBR)
- Gas-Solid Catalytic Reactions (GSC)
- Gas-Solid Noncatalytic Reactions (GSNC)
- General (GEN)
- Multiphase Reactors (MPR)
- Polymer Reactors (PR)

Tentative Technical Programme

Monday: 9 January

0845-1000 **Opening of the Conference**
Inaugural Lecture 'Early Developments in Chemical Reaction Engineering' by Prof. N.R. Amundson (Houston)

1000-1015 **Coffee**

Plenary Lectures

1015-1100 Aris (Minnesota) — Problems in the Dynamics of Chemical Reactors

1100-1145 Froment (Gent) — Progress in the Fundamental Design of Fixed Bed Reactors

Technical Sessions (Parallel)

	Session I (DYN)	Session I (GSC)
1145-1245	Takoudis (Purdue)	Gunn (Swansea)
	Takoudis (Purdue)	McGreavy (Leeds)
	Chang (California)	Cresswell (Zurich)
1245-1400		**Lunch**

Plenary Lectures

1400-1445 Hoffman (Erlangen) — How to get Reliable Laboratory Data for the Design of Chemical Reactors

Luss (Houston) — Problems in the Dynamics of Chemical Reactors

Technical Sessions (Parallel)

	Session II (DYN)	Session II (MPR)	Session II (GSC/GSNC)
1530-1730	Kapila (Rensselaer)	Speakers	Zoulalin (Campiegne)
	Malros (Novosibirsk)	to be	Narsimhan (Bangalore)
	Dogu (Ankara)	finalised	Foggler (Michigan)
	Kulkarni (Pune)		Hughes (Salford)
	George (Alberta)		Bakshi (Saskatchewan)
	Sundaresan (Princeton)		Mukhopadhyaya (Delhi)

Tuesday: 10 January

Plenary Lectures

0830-0915 Doraiswamy (Pune) — State of the Art of Theories of Adsorption on Heterogeneous Surfaces

0915-1000 Astarita (Naples) — Gas Treating with Chemicals Solvents

1000-1015 **Coffee**

1015-1100 Sharma (Bombay) — Some Novel Aspects of Liquid-Liquid Reactions

1100-1145 Boreskov (Novosibirsk) — Reactors for Reactions on Solid Catalysts

Technical Sessions (Parallel)

	Session III (MPR)	Session III (GSC)	Session III (MPR)
1145-1245	Carra (Milan)	Lakshmanan (Bangalore)	Hammer (Aachen)
	Kastanek (Prague)	—	Carberry* (Notre Dame)
	Baldi (Torino)	Acharya (Bombay)	Choudhary (Pune)
1245-1400		**Lunch**	

1400-1530 **Panel Discussion**

Technical Sessions (Parallel)

	Session IV (MPR)	Session IV (GSC)
1530-1730	Tavarides (Syracuse)	Dogu (Ankara)
	Deckwer (Oldenburg)	Rajadhyaksha (Bombay)
	Gut (Zurich)	Dadyburjor (Rensselaer)
	Joshi (Bombay)	Prasad (Hyderabad)
	Sohlo (Oulu)	—
	Chaudhari (Pune)	—

Wednesday: 11 January

Plenary Lectures

0830-0915 Shinnar (New York) — Thermodynamic Constraints in Chemical Reactors

0915-1000 Potter (Monash) — Recent Advances in Fluidization Reaction Engineering

1000-1015 **Coffee**

1015-1100 Ray (Wisconsin) — Polymerization Reaction Engineering

1100-1145 Varma (Notre Dame) — Optimal Catalyst Activity Profiles in Pellets

1145-1200 **Coffee**

1200-1245 Ramkrishna (Purdue) — Cybernetic Modelling of Microbial Cell Population

1245-1400 **Lunch**

Plenary Lectures

1400-1445 Østergaard (Lyngby) — Transport Phenomena and Reaction Kinetics in Three Phase Reactors

1445-1530 Davidson (Cambridge) — Fluidized Combustion of Coal with Reaction Engineering

Technical Sessions (Parallel)

	Session V (GEN/FBR)	Session V (PR)	Session V (BR)
1530-1730	Wakao (Yokohama)	Gandhi (Kanpur)	Blanch* (California)
	Pirard (Liese)	Anil Kumar (Kanpur)	Schugerl (Hannover)
	Varma (Saskatchewan)	Fan (Kansas)	Taguchi* (Osaka)
	Cassano (Santa Fe)	Ravindranath (Pune)	Bailey* (California)
	Grace (British Columbia)	Balaraman (Pune)	Moo-Young* Waterloo)
	Mujumdar (Montreal)		Karanth (Pune)
	Laguerie (Toulouse)		

* to be confirmed — under review

SUBMISSION OF PAPERS

- The authors should send their manuscripts before 30th June 1983 and the revised versions before 31st August 1983 to

  Dr. R.A. Mashelkar
  Secretary, ICREC
  National Chemical Laboratory
  Pune 411 008, India

- Detailed instruction sheets for preparing camera ready papers will shortly be sent to the contributing authors.
- The proceedings of ICREC will be published by Wiley Eastern and will be distributed internationally by Wiley's international network.

FACILITIES FOR LECTURE PRESENTATION

- The following facilities will be available to assist the speakers.
  - 35 mm slide projector
  - Overhead projector
  - 16 mm cine projector
  - 35 mm cine projector

REPRESENTATIVES ABROAD

Europe — Prof. G. Froment
(Laboratorium voor Petrochemische Technik,
9000 Gent, Belgium)

Canada — Prof. A. Mujumdar
(Chem. Eng. Dept., McGill Univ.,
3840 Univ. Street, Montreal,
Canada H3A 2A7)

USA — Prof. A. Varma
(Chem. Eng. Dept., Univ. of Notre Dame,
Indiana 46556, USA)

TRANSPORTATION

NCL is situated about 8 km from Pune railway station and 17 km from the airport. Transportation will be provided for delegates arriving at the airport/railway station on Saturday (January 7) and Sunday (January 8).

EXCURSIONS

It is proposed to arrange excursion tours to the world famous caves of Ajanta and Ellora and also to picturesque Goa. Details regarding the tour arrangements will soon be finalised and intimated to the participants.

ACCOMPANYING PERSONS' PROGRAMME

A programme of sightseeing to places of interest in the local region is also being organised. Details will be furnished in the near future.

ACCOMMODATION

Accommodation is available in hotels in Pune. Current rates (subject to change) are as follows ($ = Rs.10):

Category A1	Single Bed	Rs.500
Category A2	Double Bed	Rs.600
Category B1	Single Bed	Rs.150
Category B2	Double Bed	Rs.250

Category A1, A2 : 3-Star hotel with swimming pool
Category B1, B2 : Near 3-star facilities but no swimming pool

Please fill in the enclosed form and return it to the Secretary, ICREC

Brought out by
the technical services division of NCL

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INTERNATIONAL CHEMICAL REACTION ENGINEERING CONFERENCE

icrec

NATIONAL CHEMICAL LABORATORY
PUNE 411 008 (INDIA)

BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY AT NCL

Background

Taking note of the significant developments in the area of biochemical engineering and biotechnology the world over and its importance in the Indian context, this was declared to be a major thrust area by NCL. The programme began rather modestly in 1977. A biochemical engineering group was established in the division of chemical engineering, which could draw upon the expertise already built up at NCL in biochemistry, microbiology, chemical engineering and process development. The group has steadily grown over the past few years and is at present handling a number of important basic and applied projects in enzyme engineering and fermentation technology. The group has come to be recognised as an important centre of biochemical engineering in India.

Equipment and staff :

Excellent bioengineering facilities have now been established, partly through the financial assistance from United Nations Development Programme [UNDP]. The equipment already available provides bulk of the facilities for carrying out a sophisticated bioengineering programme and additional facilities to be added during 1983 will aid the programme further. A complete list is attached. The staff available consists of about a dozen members with qualifications and experience in chemical and biochemical engineering, biochemistry and microbiology.

Projects in Progress

Fig. 1 provides the details of the individual activities in a summary form.

Biochemical Engineering Science

In the area of biochemical engineering science, following are the problems being handled.

Enzyme Engineering

Kinetics of enzyme reactions, interaction of diffusion with bioreactions, deactivation and stability of bioreactors and modeling of immobilized enzyme and whole cell reactors.

Fermentation Engineering

Oxygen transfer effects in fermentors, rheological behaviour of fermentation broths and kinetics of fermentation reactions.

Biotechnology: In the area of biotechnology the problems under investigation are as follows

1) Utilization of renewable resources

Bioconversion of lignocellulosics to glucose and microbial biomass product, cellulase enzyme production in instrumented fermentors and optimization of fermentation parameters.

2) Immobilized enzyme and whole cells process development

Production of 6-APA from Penicillin-G using immobilized penicillin acylase, use of immobilized yeast cell fermentors for production of ethanol from sugars etc.

3) Fermentation process development

Production of chemicals from molasses and other sugars.

4) Pollution abatement

Biological waste treatment of distillery effluents. The work in this area is about to be initiated.

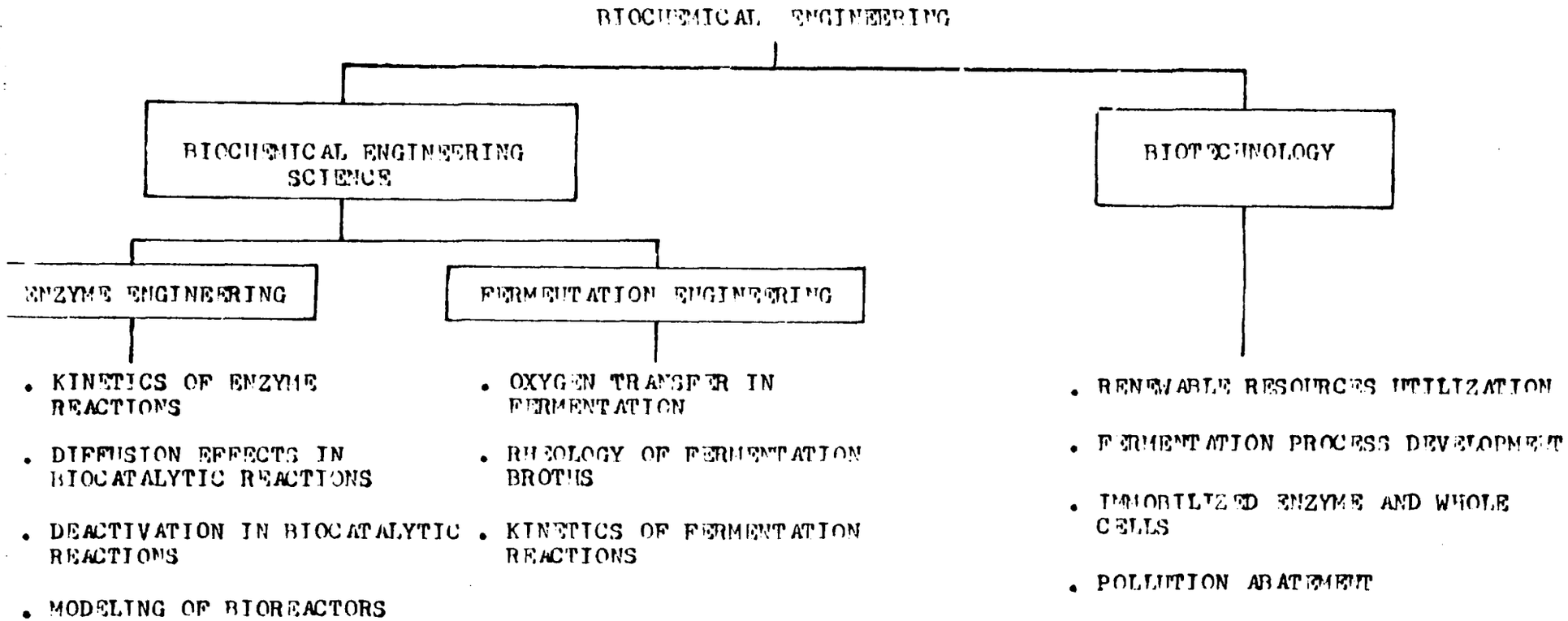

FIG.1 BIOENGINEERING ACTIVITIES AT NCL

EXPERIMENTAL FACILITIES IN THE BIOENGINEERING GROUP

1) LKB instrumented fermentor 2-4 lit. 8-12 lit. capacity
2) MBS Labroferm - A set of 3 fermentor 14 lit capacity
3) Gallenkamp minifermentor of 800 ml capacity
4) Shimatzu UV-VIS spectrophotometer, fully automatic
5) EQIL visible spectrophotometer
6) ARMIL-NUCCN gas chromatograph
7) Yellowsprings 23A glucose analyzer
8) Apple II microcomputer
9) ERGAVAAL Zeiss optical microscope
10) Basic facilities such as rotary shakers, sterile chamber, B.O.D. incubator, laboratory centrifuges, analytical balances, ovens and autoclaves.
11) CHEMAP 150 lit pilot plant fermentor with microcomputer control
12) MBS Labroferm - a set of 3 fermentors 14 lit capacity
13) BÜCHI nitrogen analyzer
14) Shimatzu Gas Chromatograph - microprocessor controlled
15) IR gas analyzer
16) Paramagnetic oxygen analyzer
17) Laboratory spray drier

*To be added in 1983

LIST OF PUBLICATIONS IN BIOCHEMICAL ENGINEERING 1978-1983

NATIONAL CHEMICAL LABORATORY, POONA - 8

1. Effect of Internal and External Diffusion on the Apparent Stability of Immobilized Enzyme Catalysts
S.S. Nair and N.G. Karanth
J. Appl. Chem. and Biotech.., 28 569 [1978]

2. Diffusional Influences on the Parametric Sensitivity of Immobilized Enzyme Catalysts
N.G. Karanth and J.E. Bailey
Biotechnol Bioeng., 20 1817 [1978]

3. Effect of Immobilized Enzyme Deactivation in Fixed and Fluid-Bed Reactors
A. Sadana
Biotechnol. Bioeng. 20 781 [1978]

4. Bubble Motion and Mass Transfer in Non-Newtonian Fluids: Single Bubble in Power Law and Bingham Fluids
S. Bhavaraju, R.A. Mashelkar and H. Blanch
AIChE J., 24 1063 [1978]

5. Bubble Motion and Mass Transfer in Non-Newtonian Fluids: Swarm of Bubbles in a Power-Law Fluid
S. Bhavaraju, R.A. Mashelkar and H. Blanch
AIChEJ. 24 1070 [1978]

6. Mass Transfer in Aerobic Fermentors
R.A. Mashelkar
Proc. Recent Advances in Fermentation, ICMA, 1979, P 25

7. Multiple Continuous Stirred Tank Reactors vs Batch Reactors - Some Design Considerations
N.G. Karanth
Biotechnol. Lett. 1 139 [1979]

8. A Generalized Optimum Temperature Operations Criterion for a Deactivating Immobilized Enzyme Batch Reactor
A. Sadana
A.I.Ch.E.J. 25 535 [1979]

9. Criteria for Multiple Steady States in Immobilized Enzyme Systems
B.D. Kulkarni and P.A. Ramachandran
Biotechnol. Bioeng. 22 1759 [1980]

10. Criteria for Multiplicity for Complex Biochemical Reactions
A. Sadana, B.D. Kulkarni and P.A. Ramachandran
Chem. Engg. Commun. 7 339 [1980]

11. A Deactivation Model for Immobilized and Soluble Enzymes
A. Sadana
Biotechnol. Lett. 2 279 [1980]

12. A deactivation Model Involving pH for Immobilized and Soluble Enzymes
A. Sadana
Biotechnol. Lett. 2 465 [1980]

13. Immobilized Enzyme Reactor with Pellicular Catalyst
N.G. Karanth and V.S. Patwardhan
Chem. Eng.J. 19 167 [1980]

14. Bioconversion of Molasses to Single Cell Protein
N.G. Karanth
Maharashtra Sugar 5 [7] 67 [1980]

15. A useful Sparger Arrangement for Fermentations Involving Heavy Mycelial Suspensions
N.G. Karanth and A.C. Manchanda
Biotechnol. Bioeng. 22 1965 [1980]

16. Use of Modified Double Reciprocal Plot for Enzyme Kinetic Parameters - A Pitfall
N.G. Karanth and A.K. Srivastava
Biochimica et Biophysica Acta 615 279 [1980]

17. Some observations on the Similarities Between Batch and Plug Flow Systems as Applied to Enzyme Reactors
N.G. Karanth
Biotechnol.Bioeng. 23, 225 [1981]

18. Analysis of Multiple Steady States of Complex Biochemical Reactors
P.A. Ramachandran, B.D. Kulkarni and A. Sadana
J. Chem. Technol. Biotechnol. 31 546 [1981]

19. Convective Diffusion in Structured Fluids : Need for New Analysis and Design Strategies
A.Dutta and R.A. Mashelkar
Rheol. Acta. 21 52 [1982]

20. Film Diffusional Influence on the Kinetic Parameters in Packed Bed Immobilized Enzyme Reactors.
V.S. Patwardhan and N.G. Karanth
Biotechnol.Bioeng.24, 763 [1982]

21. Ethanol Productivity in Immobilized Cell Bioreactors
N.G. Karanth
Biotechnol. Lett. 4, 2 [1982]

22. Studies on Fermentation Broth Rheology of a Penicillium Strain Using Cellulose as Substrate
A.C. Manchanda, V.V. Jogdand and N.G. Karanth
J. Chem. Technol. Biotechnol. 32, 660 [1982]

23. Comments on Websters' Remarks on Effect of Internal Diffusion Resistances on the Lineweaver - Burk plot.
N.G. Karanth
Biotechnol. Bioeng. 24, 931 [1982]

24. Film Resistances in Packed Bed Immobilized Enzyme Reactors: A Reassessment of the Kinetic Plots,
N.G. Karanth and V.S. Patwardhan
Biotechnol. Bioeng. 24, 2269 [1982]

25. Studies on Cellulase Production by a Penicillium Strain in an Instrumented Fermentor
A.V. Joglekar, M.C. Srinivasan, A.C. Manchanda, V.V. Jogdand and N.G. Karanth
Enzyme Microb.Technol. 5 22 [1983]

26. Significance of β -Glucosidase activity on the Measurement of Exoglucanase Activity of Celluloytic Fungi
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APPENDIX D

2 July 1983
English

RESTRICTED

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 l/day alcohol. This will entail - at the last stage of the program - construction of a semicommercial plant producing 1000 l/day alcohol at an industrial site. The output of the project at NCL amy be a complete process design and operation at 100 l/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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The Waterloo Process for SCP Production From Waste Biomass

M. Moo-Young, A. J. Daugulis, D. S. Chahal and D. G. Macdonald*

A novel fermentation process has been developed for the bioconversion of agricultural and forestry wastes into proteinaceous feed/food products. The process is based on the mass microbial cultivation of a new cellulolytic fungus, *Chaetomium cellulolyticum*, in solid-substrate systems. Preliminary feeding trials indicate that the SCP products are suitably nutritious, digestible, and non-toxic in animal feed protein rations. Economic analyses indicate that the process could be operated at a profit for a range of realistic scenarios in both developed and developing countries.

Introduction

A growing global need for protein products has led to an intensive search for unconventional agricultural sources. One increasingly attractive source is cultured microbial biomass, generally referred to as SCP (single cell protein) which can be factory-produced by fermentation processes[1-3].

Several types of fermentation processes have been proposed for the production of SCP products[4]. Notable among these are the British (ICI, BP), American (Amoco), Japanese (Kanegafugi), Finnish (Pekilo), Swedish (Symba), Russian, and Cuban processes which have been commercially realized. A major disadvantage of all these processes is their reliance on feedstocks which are now expensive, some prohibitively so (methanol, ethanol, molasses, wood hydrolysates, hydrocarbons), or are rapidly becoming scarce (waste sulphite liquor, potato wastes). In addition, the levels of the processing technologies are unattractively high especially for many developing countries where protein is needed most. The other processes which have been proposed for SCP production (e.g., GE, LSU) are not yet commercially attractive, primarily because of unfavourable process economics and/or unsatisfactory product quality.

We have developed a fermentation process which is capable of overcoming the above problems. The process utilizes raw materials which occur universally in large quantities as waste by-products. These materials include agricultural wastes such as crop residues (e.g., straw, corn stover, bagasse) and animal manures, and forestry residues such as wood sawdust and pulp-mill sludges. The process concurrently alleviates environmental pollution frequently generated by these wastes. By employing low-technology operations, the process could find appropriate applications on feedlots where the products could be used to replace the traditional soymeal and/or fishmeal as animal feed protein supplements. This application could lead to lower consumer meat prices and the release of soymeal for direct human consumption. In addition, some developing countries could use the process to transform their abundant surplus carbohydrates into much needed protein.

Several other processes, notably the Canadian (Stake), American (Jelks) and Danish (Rexen) processes, have been proposed to enhance the digestibility of various lignocellulosic materials, such as crop and wood residues, for use in ruminant feeds. However, it should be noted that these processes merely produce feed carbohydrate, which is not a scarce commodity even in developing countries, and do not convert any of the raw materials into protein[3,10].

So far, the Waterloo SCP process has been successfully tested in a 200-litre pilot fermentor; further technical improvements using a continuous-flow 1,000-litre demonstration unit are now being analysed. Satisfactory preliminary feeding trials of the products have also been conducted on rats[9]; further tests on rats, chickens and sheep are in progress. An overview of the process performance and the product quality is given below.

Process outline and innovations

The Waterloo process is based on the mass microbial cultivation of a new cellulolytic fungus, *Chaetomium cellulolyticum*[4]. As illustrated in the Figure, the process uses a three-stage operation which involves: (a) thermal and/or chemical pretreatment of a cellulosic material, (b) aerobic fermentation of the pretreated material with nutrient supplements, (c) separation of the suspended solids (the product) from the fermented broth. The cellulosic material provides the main carbon source for the fermentation. The main non-carbon nutrient supplements (N, P, K, etc.) are derived from commercially-available fertilizer blends or animal manure[7]. If manure is used, it is pretreated by anaerobic fermentation to produce methane fuel gas as a by-product which supplies operating energy[7-13].

The cellulosic raw material is pretreated with hot water or dilute alkali (NaOH or NH_3), depending on the feedstock type, to reduce the inherent recalcitrance of the solids to fermentation by swelling and/or partial delignification. The basic process is carried out as a solid-substrate fermentation in slurry systems[4]. Depending on the product requirement, a forage grade carbohydrate co-product of unfermented cellulose may also be produced admixed with the main SCP product. The co-product is rendered digestible by the action of the extracellular fungal cellulase enzymes which are generated during the fermentation[3,4]. Any residual lignin serves as inert diet roughage in the product. Because of the large mycelial growth forms of the fungus, the SCP product can be recovered by relatively simple, inexpensive filtration methods. The effluents of the process are essentially carbon dioxide and BOD-free water. Thus, an

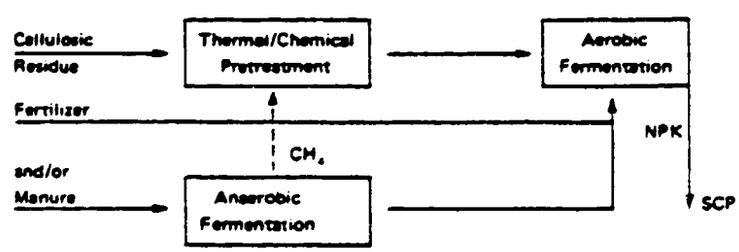

Figure: Generalized outline of the Waterloo bioconversion process for SCP production from agricultural or forestry wastes

*Prof. Moo-Young is with the Chemical Engineering Dept., University of Waterloo, Waterloo, Ontario, Canada. Dr. Daugulis is a Research Engineer, Dr. Chahal is a Research Microbiologist and Dr. Macdonald is a visiting Associate Professor in the same department.

Table 1 Typical conditions and performance of the aerobic fermenter (slurry system)

Substrate	: Corn stover
Solids Loading	: 2% W/V
Temperature	: 37°C
pH	: 5.5 (controlled)
Growth Rate	: 0.24 hr^{-1} (continuous)

Table 2 Typical conditions and performance of the anaerobic fermenter

Substrate	: Cattle Manure
Solids Loading	: 8% W/V
Temperature	: 39°C
pH	: 7 to 8 (uncontrolled)
Retention Time	: 12 days (semi-continuous)
Gas Production	: 1.2 1/1 medium/day
Gas Composition	: 65% CH_4, 35% CO_2

Table 3 Profile of essential amino acids (% DM protein) in the Waterloo fungal SCP and other protein products

Amino Acid	FAO Ref.	Soybean Meal	Waterloo SCP	Fodder Yeast
Isoleucine	4.2	4.2	4.7	5.3
Leucine	4.8	7.7	7.5	7.0
Lysine	4.2	6.4	6.8	6.7
Methionine + Cystine	4.2	2.2	2.6	1.9
Phenylalanine	2.8	4.7	3.8	4.3
Threonine	2.6	3.6	6.1	5.5
Tryptophan	1.4	1.7	N.A.	1.2
Tyrosine	2.8	2.7	3.3	3.3
Valine	4.2	4.4	5.8	6.3

Table 4 Typical compositions (% DM) of the Waterloo fungal SCP and other protein products

Component	WAT-SCP	Soymeal	Yeast
Protein	46%	46%	45%
Carbohydrate	35%	42%	33%
Fat	10%	6%	6%
Nucleic Acids	5%	low	8%
Minerals, Ash	5%	7%	5%
Vitamins	High	Low	High

additional benefit of the process is abatement of the environmental pollution normally caused by the feedstocks used.

Table 1 shows some typical operating conditions and performance of the aerobic fermentor for the utilization of corn stover and fertilizer mixtures. The specific growth rate of 0.24 hr^{-1} is one of the highest known for cellulolytic fungi[6]. Typical results for the anaerobic fermenter utilizing cattle manure are given in Table 2.

Various additional inventions have been developed to improve the basic Waterloo process. For example, productivity can be enhanced by using a polymer additive in the fermentation medium[9], a tubular fermentor design[5], semi-solid systems[10], and a mixed culture. All the inventions are protected by patents allowed or pending; permission for their commercial uses must be obtained from the University of Waterloo.

Product quality

The profile of the essential amino acids for the Waterloo fungal SCP is given in Table 3 which indicates that its protein nutritional value is comparable with a well-known fodder yeast, soymeal, and the FAO reference standard (for proper human nutrition). Table 4 gives the overall compositions of the Waterloo SCP, soymeal and the yeast. Although SCP products are often compared with soymeal, it should be noted that they are more closely related to meat in terms of not only protein but also the spectrum of other important nutrient components such as fats and vitamins, which are often low in processed commercial-grade soymeal. It should also be noted that the Waterloo SCP is more attractive than the yeast for direct human use because of its lower content of nucleic acids and higher content of sulphur-containing amino acids.

Table 5 gives other typical properties of the Waterloo SCP product, which now appears to be satisfactory as an animal feed protein ration in terms of safety, digestibility and nutritive value. The physical properties of the dried product also appear to be suitable for marketing purposes.

Table 5 Typical properties of the dried mixed fungal SCP product obtained from straw crop residues

Colour	: Grey
Texture	: Granular
Odour	: Mushroomy
Moisture	: 8%
Digestibility	: 73% NFE (Rats)
Biomass Content	: 73% DM
Cellulose Content	: 4% DM
Lignin Content	: 12% DM
Ash Content	: 12% DM

Process economics

The process costs are minimized by using cheap raw materials (actually, waste residues), low-technology conditions (e.g., rubber-lined ditch fermenters; low temperatures and pressures) and efficient mass and energy exchanges between processing streams. The return on investment (ROI) before taxes, and the discounted cash flow return (DCFR) after taxes, can be readily derived for various scenarios from computer simulation programs which we have developed for the process. For example, Tables 6 and 7 give the predicted minimum economic plant sizes for some low-risk (DCFR < 20%) and high-risk (DCFR > 20%) assumptions. The tables show that the process can be operated at a profit to upgrade crop residues and forestry residues for a range of practical conditions. These examples of economic analyses are based on current (April 1979) Canadian figures for the selling price of spent brewers' yeast (used as a comparison product) and the cost of straw (used as a typical crop residue), and for typical disposal charges for Kraft pulpmill sludges (in Ontario). Plant amortization is conservatively rated at 10 years.

For the bioconversion of agricultural wastes, the process is particularly attractive for a farm co-operative venture. For example, since a typical 300-acre Ontario crop farm produces a yearly average of about 180 tonnes of waste straw and/or corn stover, only about three such farms would be required to operate a profitable central processing co-operative. Tables 6b and 6c show that the economics would improve if fertilizer is replaced with anaerobically predigested animal manure as the source of the NPK supplements, and the methane fuel gas which is concurrently produced is used in the process. In Table 6b just enough NPK is produced by the anaerobic digestor to meet the requirements for the SCP product synthesis. In Table 6c, all of the fuel gas requirements are met by the methane from the anaerobic digestor resulting in an excess supply of NPK fermentation supplements which are credited, as soil fertilizer by-products, to the process. As shown in Table 6d, the potential process economics shown in Table 6b are further improved for a feedlot scenario of onsite matched continuous production and consumption of an undried product, as feed slop, since the product drying and bagging costs are avoided.

For the bioconversion of forestry residues, Table 7 summarizes the profitability of the process in the upgrading of a pulpmill waste sludge. This application is very attractive in Canada and the U.S. since the process can be credited with the usual high charges for legally-enforced environmentally acceptable methods of disposing the waste sludge. It is seen that a minimum economic plant size would require about 10 to 20 tonnes of sludge daily which is the range produced by typical Kraft pulp mills. Clarifier equipment made redundant by the process is not credited in these calculations.

Table 6 Estimated minimum economical plant sizes for the bioconversion of agricultural wastes (e.g., corn stover, straw, manure) into WAT-SCP quantities expressed on dry weight basis in tonnes/day (t/d).

(a) NPK from fertilizer. Dried product containing 73% DM SCP

DCFR (%)	ROI (%)	Product (t/d)	Straw (t/d)	Fertilizer (t/d)	Capital ($)	Operating ($ / yr)
10	18	1.9	2.6	0.13	310,700	228,200
20	29	2.5	3.5	0.18	374,300	268,900
30	41	3.6	4.9	0.25	465,500	332,000

(b) NPK from manure anaerobic digester to meet SCP product synthesis requirement. Dried product containing 60% DM SCP

DCFR (%)	ROI (%)	Product (t/d)	Straw (t/d)	Manure (t/d)	Capital ($)	Operating ($ / yr)
10	18	1.9	1.9	3.6	316,300	194,100
20	29	2.5	2.5	4.8	379,900	222,700
30	41	3.5	3.5	6.6	469,300	265,700

(c) NPK from manure anaerobic digester to meet CH_4 process fuel gas requirement and credit excess NPK. Dried product containing 60% DM SCP.

DCFR (%)	ROI (%)	Product (t/d)	Straw (t/d)	Manure (t/d)	Capital ($)	Operating ($ / yr)
10	18	1.8	1.8	3.6	286,400	184,400
20	29	2.3	2.3	4.7	339,600	208,400
30	41	3.2	3.2	6.4	414,900	244,600

(d) NPK from manure anaerobic digester (scenario in Table 6b). Undried, filtered product containing 60% DM SCP

DCFR (%)	ROI (%)	Product (t/d)	Straw (t/d)	Manure (t/d)	Capital ($)	Operating ($ / yr)
10	18	1.7	1.7	3.2	266,600	178,900
20	29	2.2	2.2	4.1	312,900	200,200
30	41	3.0	3.0	5.5	378,500	232,000

Table 7 Estimated minimum economical plant sizes for the bioconversion of forestry wastes (e.g., Kraft pulpmill clarifier sludge) into WAT-SCP. NPK supplements from fertilizer. Product contains 56% DM SCP.

DCFR (%)	ROI (%)	Product (t/d)	Sludge (t/d)	Fertilizer (t/d)	Capital ($)	Operating ($ / yr)
10	17	3.1	4.3	0.12	639,400	299,900
20	28	4.8	6.6	0.19	837,500	388,500
30	40	8.0	11.0	0.32	1,159,200	548,000

Tables 6 and 7 also give estimates of the expenditures, both capital and operational, which are required for the manufacture of the WAT-SCP product from agricultural wastes, at various profitability levels. For a farm cooperative scenario, the profitability should be higher since some of the anticipated costs, e.g., for marketing, would be avoided (see Table 8). As expected, these expenditures are relatively small compared to the range of expenditures required for other SCP processes which use expensive feedstocks and high-technology operations.

Concluding remarks

It should be noted that the governments of many countries usually provide tax incentives for new technological developments such as the present one. These credits have not been included in the above calculations. In addition, application of the present process in some countries would allow the conservation of their much-needed foreign currency reserves by promoting the use of indigenous raw materials and labour, and the reduction of cash outlays for protein importation.

It should also be noted that the above calculations are given for the basic generic Waterloo process only. The potential process economics are even more attractive if the productivity enhancements are considered when using the other innovative developments, e.g., mixed cultures, tubular fermenter design, polymer additives, and solid-state systems. Various sensitivity analyses of the economic predictions from our computer program are now being used to identify optimal conditions for a wide range of realistic scenarios. In addition, these predictions are being compared with the potential process economics for the bioconversion of agricultural and forestry wastes into two other types of products, methane fuel gas and fuel-grade alcohols, as energy sources.

Table 8 Breakdown of annual costs for scenario in Table 6b (agricultural wastes) yielding 20% DCFR

Raw Materials	14.5%
Utilities	11.3%
Labour	22.9%
Fringe Benefits	9.2%
Support Costs	16.1%
Marketing Costs	11.6%
Depreciation	14.4%

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APPENDIX F

IMMOBILIZATION OF YEAST CELLS ON VARIOUS SUPPORTS FOR ETHANOL PRODUCTION

M. Moo-Young*, J. Lamptey and C.W. Robinson

Biochemical and Food Engineering Group
Department of Chemical Engineering
University of Waterloo
Waterloo, Ontario, Canada N2L 3G1

ABSTRACT

An immobilization technique has been developed for a packed bed fermenter which is being considered as one stage of a process for the production of fuel-grade ethanol from sugar solutions. Relatively inexpensive beech wood chips have been successfully used as the support material and relatively high cell loadings of 188 mg DW cells/g DW support have been achieved for a test system of Saccharomyces cerevisiae cultures.

No washout of adsorbed cells occurs below a superficial liquid velocity of 8.9×10^{-2} cm/s which can be increased to 9.7×10^{-2} cm/s by including up to 1% Hercofloc solution in the reactor medium during the immobilization procedure. The immobilization procedure is practically unaffected by pH and temperature in the range 3.5 to 5.0 and 22°C to 37°C respectively.

Typical ethanol productivity of 21.8g/l·hr has been obtained with wood-chip-adsorbed cells, which compares well with optimal values of 18 to 32g/l·hr obtained using free-suspension cultures in stirred-tank fermenters with cell recycle.

KEYWORDS

Immobilized yeast cells; packed-bed reactor; cell loading; critical flow velocity; wood chips; ceramic.

INTRODUCTION

Over the past few years, the growing concern about the energy crisis, which was caused by the shortage of petroleum and the resulting increase in the price of gasoline, has created a surge of interest in alternative energy sources, especially renewable ones (Sitton and colleagues, 1979; Wick and Popper, 1977; Chose and Tyagi, 1979; Cysewski and Wilke, 1976; Larsson and Mosbach, 1979). The production of ethanol with improved biotechnology has been viewed with particular interest since it can be used directly as fuel in existing motor vehicles. However, significant improvements in ethanol production technology are necessary in order to substantially reduce the overall base cost of production.

Continuous flow, stirred reactors which lose microorganisms in the effluent are limited in throughput by cell growth rates. Immobilized cell reactors are not

limited (to the same extent) by cell washout and relatively high cell densities and throughput rates are possible. Consequently, this type of reactor is expected to give a higher volumetric fermentation capacity with possibly superior performance economics.

Little quantitative information is available regarding the key factors (particle size, pH, temperature and the critical flow velocity capable of creating hydrodynamic forces sufficient to detach the cells from the support) which influence the immobilization procedure and, hence, the operation of an immobilized yeast cell reactor for ethanol production. The purpose of this paper is to report the results of a quantitative investigation of those factors which influence the degree of cell loading (mg DW cell/g DW support) obtained during the immobilization step. Primarily, we have studied the immobilization of S. cerevisiae by adsorption on beech wood chips.

MATERIALS AND METHODS

Experimental Equipment

The packed-bed reactor used for the immobilization studies consisted of a jacketed glass column 0.8m in height and 4.7×10^{-2}m i.d. Sample withdrawal ports were located along the height of the column at 11.5×10^{-2}m intervals.

During immobilization, a concentrated cell suspension (10 to 15 kg DW/m^3) was circulated from a surge tank through the packing in upflow. The surge tank was equipped with a temperature indicator-controller by means of which the contents were maintained at the desired test temperature. The column reactor together with the support material and connecting tubes was pre-sterilized in an autoclave at 121°C for 30 min.

Microorganism

Saccharomyces cerevisiae NRRL Y-132 was obtained from the NRRL, USDA (Peoria, Illinois). The culture was maintained on malt extract-yeast extract-glucose-peptone (MYGP) slants. Prior to experimental use, cells were grown aerobically for 24 hrs in a shaker incubator at 30°C in 250ml flasks containing 100ml of medium containing 1% glucose, 0.5% peptone, 0.3% yeast extract and 0.3% malt extract (pH 5.0). The concentration of cells was increased to 10 to 15g DW/l by batch centrifugation.

Supports

(a) Wood chips (beech) were obtained from Abitibi Paper Company Ltd., Sheridan Park, Ontario.
(b) Ceramic (Diatomite) was obtained from Eagle Pitcher Industries Inc., Cincinnati, Ohio.

The beech wood was used in the form of chips of various sizes. The chips were initially heat treated in boiling water for 4 hours followed by soaking in 10% (v/v) ethanol for 2 hours and in boiling water for another 1 hour to remove water-and alcohol-soluble compounds from the wood.

Analytical

The concentration of suspended cells was measured either by turbidimetry or by a gravimetric method. The optical density of the cell suspension was measured at 600 nm in a spectrophotometer (Turner model 330). During fermentation, ethanol concentration was determined by gas chromatography (Hewlett-Packard F&M Scientific 700). A 1.22m by 6.25 x 10^{-3}m o.d. column packed with Porapack Q (100-120 mesh) was used with a flame ionization detector. Both the injector and the detector were kept at 170°C and the column oven operated isothermally at 150°C. Helium was used as the carrier gas at a flow rate of 30ml/min.

Immobilization Procedure

During immobilization, the concentrated suspension of cells was recirculated through the packed column for about 12 hours at a superficial velocity of about 1.7 x 10^{-2}cm/s. The column was then allowed to stand for 12 hours. In order to determine the cell loading (mg DW cells/g DW support), the circuit and support were washed by passing 0.1% glucose solution through the column (once-through mode) in order to remove the non-adsorbed cells. The liquid velocity was then increased to 1.0cm/s in order to strip off the cells from the support. The dry weight of cells stripped off the support was then measured. The above procedure was repeated for various particle sizes and for different pH's and temperatures. The time course required for the immobilization was determined by monitoring the optical density of the cell suspension in the feed tank with time during the recirculation of the cell suspension through the column. Maximum cell loading was considered to have been achieved when the optical density of the cell suspension in the surge tank reached a constant minimum value for a period of at least 2 hours.

Critical Sloughing-off Velocity

Following cell immobilization by adsorption, cell-free medium was passed through the column in a once-through, upflow mode at progressively increasing superficial velocity. The optical density of the column effluent was monitored. The liquid superficial velocity at which an appreciable rise in the effluent cell concentration was first observed was taken as being the minimal value capable of creating hydrodynamic forces sufficient to detach the cells from the support, i.e., the critical sloughing-off velocity.

RESULTS AND DISCUSSION

Cell Loading

The effects of pH, temperature and particle size on cell loading onto wood chips are shown in Table 1.

TABLE 1 Effect of pH, Temperature and Particle Size on Cell Loading (Wood Chips)

Equivalent Particle[1] Size, m x 10^3	pH	Temperature °C	mg DW cells / Specific Surface area of Support mg/m	mg DW cells / g DW Support
2.12	4.5	28	2.06	65
	4.5	37	2.10	66.7
1.28	4.0	30	4.17	163.2
	5.0	30	4.07	161.0
	5.0	22	3.98	157.9
1.22	4.0	30	4.25	188

It is evident that there is little effect of pH and temperature on the amount of cells adsorbed in the range 4.0 to 5.0 and 22°C to 37°C, respectively. However, as the size of the support [1] is decreased from 2.12 x 10^{-3}m to 1.2 x 10^{-3}m, the cell loading increases significantly from about 70mg DW/g DW support to about 190mg DW/g DW support. This increase in cell loading is due to increased availability of surface for immobilization as shown by the figures for the mg DW cells adsorbed per unit specific surface area of support. The cell loading onto the ceramic support is shown in Table 2.

TABLE 2 Effect of Temperature on Cell Loading (Ceramic Support)

Particle Size m x 10^3	pH	Temperature	mg DW cells / g support
2	4.5	28	14.2
	4.5	30	16.2
	4.5	37	14.8

[1] The equivalent particle size is defined as the diameter of a sphere with the same surface area as the chip. It is given by $d_p = w[d_c l_c + 0.5\, d_c^2]^{0.5}$ (Satterfield, 1970), where w is the shape factor, d_c is the diameter of the chip and l_c is the length of the chip.

From Table 2, the data for cell loading show that there is little effect of temperature in the range 28°C to 37°C. Comparing the results of Tables 1 and 2, cell loading onto wood chips is seen to be significantly higher than for the ceramic support. This is in agreement with the findings of Durand and Navarro (1978) who also found that the cell loading varies markedly for different supports.

Effect of Liquid Flow Rate

Figure 1 shows a typical time course of immobilization.

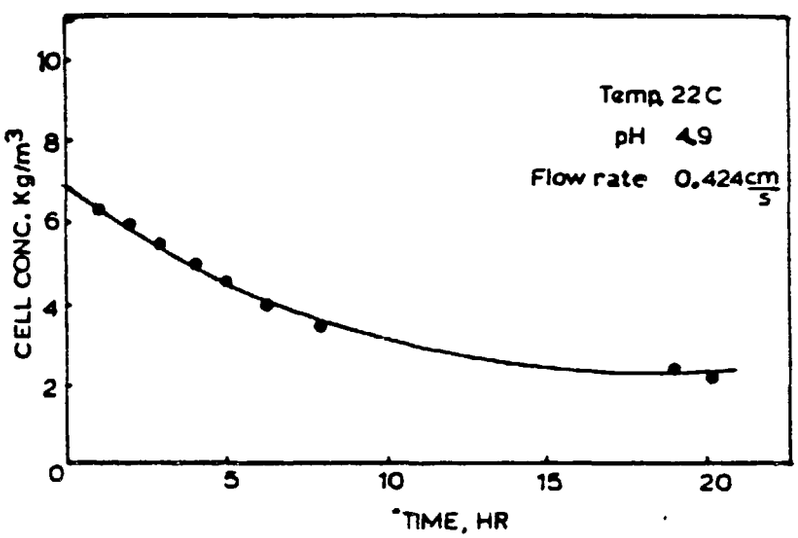

Figure 1. Decrease in free-suspension cell concentration in surge tank during immobilization procedure.

As can be seen from Fig. 1, the immobilization is time dependent. Generally, a period of 10 to 12 hrs is required for the adsorption to become fully established.

Figure 2 shows that the adsorption of yeast cells onto wood chips is a reversible process. As the superficial flow rate of the cell suspension is increased from 0.086 cm/s to 0.424 cm/s, all the cells are practically stripped off the support in a period of less than 2 hours, mainly in this case because sufficient time was not allowed for the immobilization to become fully established. When the flow velocity was then decreased to 0.086 cm/s, and maintained at this velocity for 10hrs, the cells become readsorbed and only a smaller percentage of cells become stripped-off the support when the flow velocity was once again increased to 0.424 cm/s.

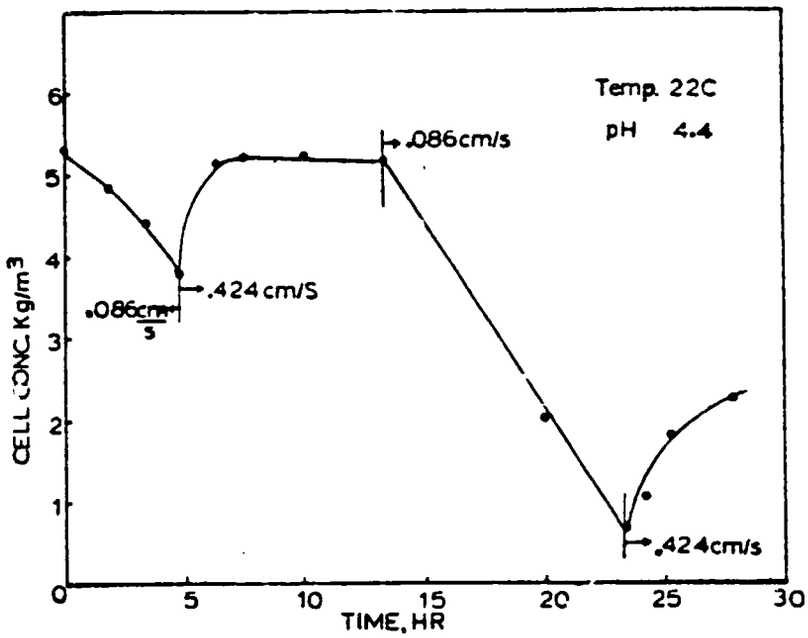

Figure 2. Effects of liquid flow velocity on cell retention in packed bed. Free-suspension cell concentration measured in surge tank during recirculation mode operation. Increase in velocity (at 5 and 23 hours) to above the critical sloughing-off velocity strips cells from support. Cell desorption is reversible; velocity reduction to normal operating range (at 13 hours) results in re-adsorption.

It is important to determine the critical sloughing-off velocity in a packed column of adsorbed cells since liquid velocities greater than the critical velocity will result in a significant amount of cells being washed off the support. Values of the critical sloughing-off velocities are shown in Table 3.

TABLE 3 Critical Sloughing-off Velocities

Support	pH	Critical Liquid Superficial Velocity cm/s x 10^2
Wood chips	3.5	8.83
	4.0	8.83
Ceramic	4.0	9.17

It is to be noted that these velocities are significantly greater than the terminal setting velocity of the cells which is on the order of 1.0×10^{-6}m/s.

By recircling up to 1% Hercofloc™ (a flocculant) solution through the packed column after immobilization, the critical sloughing-off velocity was found to increase, as shown in Table 4.

TABLE 4 Effect of Hercofloc Recirculation Through the Packed-Bed Reactor (Wood Chips)

Hercofloc Concentration (w/v)%	Critical Liquid Superficial Velocity cm/s x 10^2
0.0	8.83
0.2	9.42
0.5	9.30
1.0	9.72

Ethanol Production

Preliminary tests have been conducted using the immobilized yeast cell, packed bed reactor to produce fuel-grade ethanol from a glucose medium. A typical preliminary ethanol productivity of 21.8g/l·hr has been obtained with an effluent ethanol concentration of 60.24g/l using wood chips as the support.

CONCLUSIONS

An immobilization technique has been developed for a packed bed fermenter which is being considered as one stage of a process for the production of fuel-grade ethanol from sugar solutions. Relatively inexpensive wood chips have been successfully used as the support material and relatively high cell loadings have been achieved for a test system of glucose/yeast cultures.

Typical preliminary ethanol productivity of 21.8 g/l·hr has been obtained which compares favourably well with optimal values of 18 to 32 g/l·hr obtained for free-suspension cultures in stirred-tank fermenters with cell recycle (Ghose and Tyagi, 1979; Cysewski and Wilke, 1976). No washout of cells occurs below a superficial liquid velocity of 8.9×10^{-2}cm/s which can be increased to 9.7×10^{-2}cm/s by recirculating up to 1% Hercofloc solution in the packed-bed reactor during the immobilization procedure. The immobilization procedure is practically unaffected by pH and temperature in the range 4 to 5 and 22°C to 37°C, respectively.

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