OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

FAIR USE POLICY

CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

January 1986

RESTRICTED

ENGLISH

India.

BIOSCIENCE AND ENGINEERING

DP/IND/80/003

INDIA

15298

Technical Report *

Mission 2 to 15 April 1985

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 Mr. Gerard Goma
Consultant on Biotechnology
Conversion of Glucose to Ethanol

United Nations Industrial Development Organization
Vienna

* This document has been reproduced without formal editing.

TABLE OF CONTENTS

Abstract

The project Bioscience and Engineering DP/IND/80/003 has been reviewed on the subject concering ethanol production from molasses with immobilized cells. NCL has selected an immobilized yeast system (calcium alginate) and the screening period is finished. More optimization on this immobilized biocatalyst must be done (size, strength, durability). More efficient strains (Saccharomyces cerevisiae Y_7 and Y_{10}) must be tested in an immobilized bioreactor.

Research on enhancers is going ahead with 10 1/day reactor; they must be tested continuously.

Assays performed in 10 litre reactors indicate that it is possible to improve both rationale aeration and rationale hydrodynamics. The shape of the bioreactors, the means of their association (multistep reactor with divided feed), the method of biocatalyst utilization must be improved. Tracer technique should be used for characterizing the hydroulic behaviour.

The reactors built previously can be modified to start quickly with an extrapolable reactor.

The laboratory is now well equipped for carrying out biotechnological research. The overall impression is that the project of glucose conversion to ethanol is progressing satisfactorily and that NCL will succeed in establishing an efficient process for ethanol production by fermentation.

Activities Accomplished

- Briefing in Vienna - Dr. M. MAUNG: Technical reports of other experts were supplied by UNIDO

- Briefing in New Delhi - Dr. KAMMAL HUSSEIN

- Dr. SRINIVASAN, Dr. C. SIVA RAMAN in Poona supplied me with internal reports, organized visits to individual scientist groups, provided means to obtain overall impression of expertise and facilities at NCL for biotechnology research in the field of ethanol fermentation.

- Two meetings with the engineering groups were organized.

- One general meeting was organized at the end of the mission.

- The expert was asked to give two seminars at NCL; the topics were:

  1. Strategies in biotechnology based on economical and technical basis.
  2. New trends of ethanol fermentation based on the integration of chemical engineering and physiology.

1. Introduction

Ethanol is an inexpensive bulk chemical with a high energy value. In recent times, interest has been shown in the biological production of ethanol for use as a fuel or chemical feedstock. Since the second world war the biological production of ethanol has not been able to compete with chemical synthesis via the hydration of ethylene. The oil crisis of 1973 encouraged the re-examination of fermentation as an economic means of ethanol production particularly in countries which have large surplus of agricultural by-products such as molasses.

Profitable production of bulk chemicals by biological processes is a difficult business and ethanol is no exception to this. The cost of the carbon substrate represents 2/3 of the cost of the product. Therefore, fluctuations in raw material prices can make the biological route incompetitive. The next main cost factor, which is intrinsic to the process, is the price of steam needed to distill the product.

Efforts to develop an industrial process for the biological production of ethanol must adequately address the following criteria which are essential if the process is to be technically and economically viable:

- continuous culture
- high yields
- low residual substrate concentration
- high ethanol concentration
- high productivity

The work performed at NCL is based on this approach.

2. Reviews of the present state of studies

In India the price of ethanol is 1,1 Roupie/l ($0.1/l), molasse is 50 Roupies/t ($5/t). Due to the cost of ethanol extraction the main problems are:

- How to obtain a favourable energy balance?
- How to minimize the investment cost?

Continuous processes permit a rational utilization of energy for ethanol extraction. However, high productivities are required. To reduce the investment cost the process must be technically simple.

The objective here is to reuse the cells and increase their concentration by immobilizing them in an open pore matrix.

3. **The biocatalyst** (immobilized cells)

Several options were considered:

- yeast immobilization into gelatin: long-term stability is not good.
- yeast immobilized into agar: operational performances decline after 8 days.
- flocculated cells: some yeasts are isolated but not tested yet efficiently, with molasses it seems that there is a deflocculation (this way is not sufficiently studied).
- porous alumina bed: productivities are not sufficient even with the use of flocculant yeast.
- wood chips: productivity is weak.
- Ca algine beads: this last solution gives the best results. Stabilities of three months are performed with molasses with 1 mm pellts. This last technique must be used for future. Improvement should be performed for an efficient process.

Comments

Many works on ethanol fermentation are published on synthetic media but do not take into account the reality of the raw material like sugar cane molasses which is rich in salts. NCL works on actual raw materials.

- Because it is the calcium alginate which is insoluble in water, the stability of calcium alginate beads is connected to the calcium concentration. If there is removal of calcium (ionic exchange, salts reactions and reassociations (calcium phosphate as suggested by Dr. LINKO), beds are not stable. More of them, salt precipitations on the alginate bead can change their permeability, activity and density.

- During fermentation if beads are destabilized it will appear an increase of turbidity, a non-regular granulometry and a change in loading rate in the reactor. It seems interesting to measure on line this loading rate and to consider this as a parameter to eventually correlate with the stability of size bead.

- The problem of molasse purification is more actual - cf. acid precipitation and filtration (or centrifugation), preclarification must be solved out of the reactor even by a flocculation.

- Size pellets: Discussions around this point must be made at the light of experiments: What is cell viability inside a pore in relation with an aeration rate? Cytocolorimetry must be performed with pellets of 1, 2, 5 mm. It seems to me, that,if fluidization is performed correctly, due to O_2 low concentration pellet size lower than 2 mm preferable.

4. The preparation of biocatalyst

Two possibilities are under discussions:

1) Cells are pre-grown, centrifuged, washed, mixed with sodium alginate and precipitated in $CaCl_2$

2) Some cells are pre-grown, mixed with sodium alginate, precipitated and a growing phase is performed inside the calcium alginate.

In the first method cells are lost when centrifuging and washing. The second method, suggested by Bulock, seems more attractive but good growth needs high oxygen transfer.

Comments

The growing phase of yeast must be considered in every case as a S.C.P. problem: exactly as a "bakers' yeast process". It is necessary to avoid excess of sugar and limitation by oxygen (2 grammes of sugar and 1 gramme 02 give 1 g yeast.). In the first case, if cell growth is not optimal it will be possible to make several fermentations to produce the amount of cells required. In the second case, if the fermentation is not performed correctly, the cell concentration will be weak and the transient time to reach the steady state will be longer. With alginate beds, mass transfer is lower and limitations are stronger.

The first method seems the more efficient: cells should be concentrated by ultrafiltration - centrifugation.

5. The oxygenation problem

Alcoholic fermentation is anaerobic, but the need for oxygen appears to maintain good performance. Dr. Linko suggests a feed rate of 0.1 volume minute. The magnitude is correct, but this must be studied more accurately and connected to physiological effects.

Comments

- Gas flow range in the field of 0.05 vvm must be tested.
- Most of all, $k_L a$ value must be sufficient, at least more than 15 h^{-1}.
- Sequential aeration must be evaluated to avoid the increase of ethanol evaporation by gas. Cycles, for example, of 1 hour of aeration for 3 hours without, must be tested. the viability will be determined.
- Effect of aeration on fluidization and homogenization must be evaluated by coloured visualisation and trace techniques measurements of viability are necessary. These studies must be connected with caracterisation of residence time distributions in the bioreactor.
- Gas-lift devices are convenient for these problems and should be considered.

6. Hydrodynamic problems

Three reactors in series are constructed by NC.: these reactors have a ratio H/D = 1.5 (H = hight, D = diameter), they can be used in series or in parallel. The idea is convenient but it seems that the shape would be better if the bioreactors were cylindroconical (cylinder H = D, hight of the cone in lower position 1 D).

Recommendations and comments

For the extrapolation of the laboratory experience the reactors used are geometrically homothetic, however, extrapolation to the needs of industrial plant mixing phenomena are too complicated for this shape. The shape of the reactors is not suitable: auto-mixing bio reactors (cylindrico conical reactors with gaz-lift devices as described in the scheme 1) are superior. Due to the CO_2 produced and the shape, mixing, homogeneity of temperature, concentration is obtained more easily than with an external mecanical device. Dr. Siva Raman has suggested the addition of a decantor to the bottom to clear the molasses.

Essays on 20-30 l reactors can be achieved.

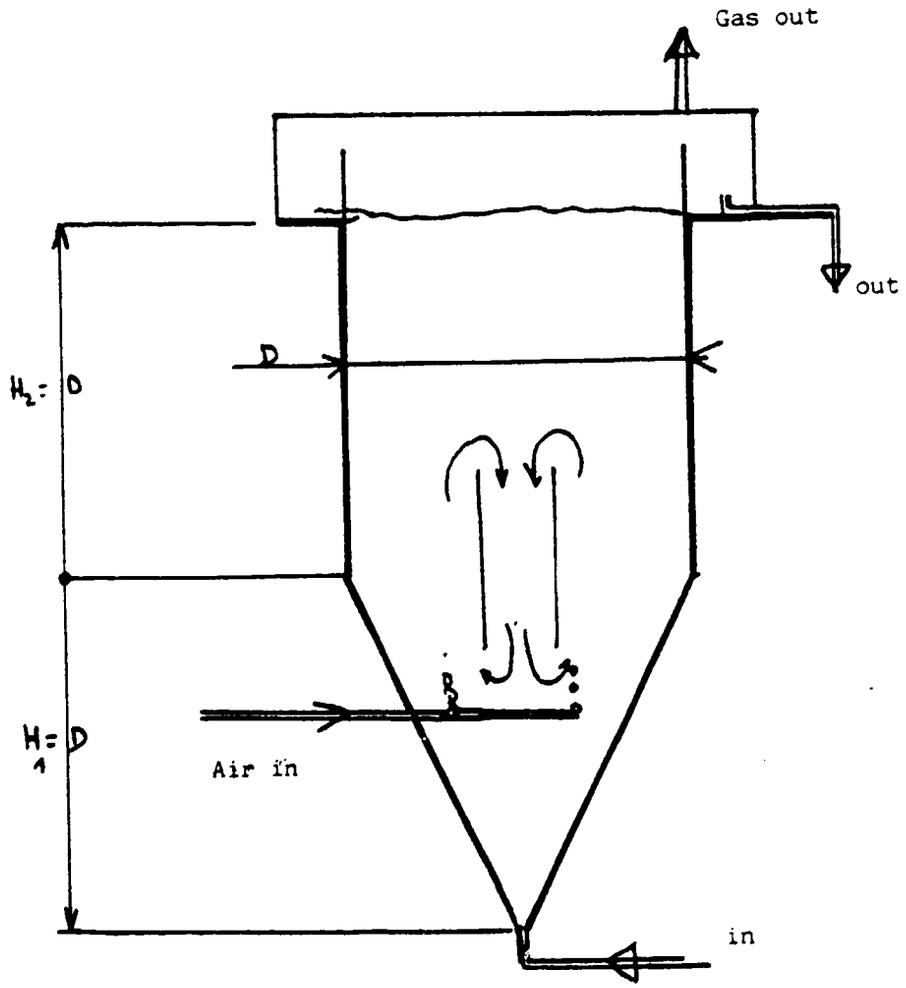

Schéma 1: the proposed bioreactor "auto mixing".

CONCLUSIONS

The team has a strong interest in the project and important work is being performed. Very good research is now in place and NCL will achieve success. Doctors Srinivasan and Siva Raman are going ahead efficiently and the programme is well managed with good interactions between engineers and microbiologists.

My recommendations and suggestions are the following:

- Pretreatment of molasses must be performed (acidification - floculation and/or filtration).
- Improve the screening of yeast: evaluate more accurately yeast Y_{10} and Y_7.
- Consider as an S.C.P. operation the initial cell growth before including it in the alginate, concentrate them by ultrafiltration (or centrifugation).
- The bioreactor should have a different shape: cylindro-conical with, at the bottom, in the conical volume a gaz-lift device for mixing.
- Optimize aeration on the basis of the new shape ($K_L a$ 15 h^{-1}, rate aeration 0.05 - 0.15 vvM).
- Improve the biogaz production on vinasse to increase the energy production.

ACKNOWLEDGEMENT

Arrangements and facilites of the NCL group were excellent.

I want to thank:

- UNIDO authorities for the excellent organization of this mission
- NCL staff for their excellent hospitality.

Dr. Mrs. Siva Raman, Dr. Siva Raman, and Dr. Srinivasan for their assistance, their warm hospitality associated with an efficient organization of my time.