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INDIA

CONSULTANCY REPORT

Prepared for the Government of India
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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

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General section

# Introduction

This report considers the status of two related UNDP-sponsored projects under the heading "Bioscience & Engineering" [IND/80/003] carried out at the National Chemical Laboratory, Pune (India) and concerned with:

(1) cellulose biotechnology

(2) ethanol technology.

Within IND/80/003 these two projects are combined with a third topic, controlled-release formulations, with which aspects this report is not concerned. The report is based directly upon discussions carried out between November 1 and November 18, 1987, in Bombay and in Pune, and upon written reports from NCL made available or undergoing preparation during that period, combined with infomation and views deriving from this consultant's previous visits to NCL under the same programme.

Because this report is in essence a terminal account of this programme and is therefore more or less simultaneous with the submission of detailed technical reports by NCL on the content and results of the programme in all its aspects, the present report does not contain detailed technical consideration of the ongoing work at NCL. Such consideration was as a matter of course carried out during the 1987 visit, and our technical discussions then resulted in a number of recommendations which are being followed by NCL in their continuation of the work, but they are not considered as being especially relevant to the present report, which concentrates on summarizing the results, benefits, and lessons both positive and negative which can be drawn from the two listed aspects of the programme. For the same reasons this report does not document in detail such matters as lists of scientific publications or of training and career development of personnel, since these will be fully documented in NCL's own reports, draft versions of which have already been discussed by this consultant as part of the study; however these are important aspects which will be referred to here in general terms where appropriate.

## Logistic aspects

On this occasion there were very few logistic problems connected with the consultant's visit. Transportation presented few problems, and all transportation in and around Pune was very efficiently provided by NCL. The officers and staff of NCL provided every facility with promptness and efficientcy throughout.

The only problem, which could have been very serious, was the failure of the Indian High Commission in London to process the requisite visa application with the expedition which had been expressly requested and which would have been appropriate for this UNIDO assignment; in fact the IHC actually "lost" the visa documentation and the situation was only retrieved after prolonged and insistent telephone calls.

**I recommend that where UNIDO business underlies a visa request that there should be some agreed and authoritative mechanism to ensure that such requests are promptly and efficiently processed.**

cellulose biotech--nology

## cellulose/cellulase biotechnology

### Overall concept

The overall concept of this part of the project centred upon three propositions, which may be stated as follows:

(1) Countries such as India have a major accessible low-cost resource in the form of lignocellulosic materials which is at present wasted;

(2) The optimum route for utilising lignocellulose is by hydrolysis followed by fermentation of glucose recovered from the hydrolysate;

(3) The optimum means of hydrolysing lignocellulose to obtain glucose is by the application of cellulase enzymes.

It is a matter of record that in this consultant's considered opinion all three of these propositions are incorrect, and it is a matter of observation that a similar judgement has been eventually reached by the workers on this project at NCL.

Without going into excessive length on these matters the objections to these propositions, and hence to the entire conceptual base of this part of the project, can be summarized as follows:

(1) India does not in fact have surplus *accessible* lignocellulose wastes since all such "wastes" are in fact in very considerable demand for direct uses as fuel, construction materials, or animal feed; in fact India suffers from a shortage of such materials not a surplus, and the only local surpluses are those which result from transportation problems, i.e. problems of accessibility.

(2) The unique polymer properties of cellulose and of the composite polymer lignocellulose provide the most advantageous basis for the exploitation of these materials as intact or modified polymers of unique properties and manifold uses, with a substantial base in traditional or recent technologies, while the best sources of fermentable sugars are on the one hand sugar-producing plants such as cane or sweet sorghum and on the other plants which produce the starch polymers which are uniquely adapted for hydrolysis.

(3) Where the hydrolysis of lignocelluloses is nevertheless a valid objective, technologies for non-biological hydrolysis have been available for many years, have been enormously improved in recent years, and are uniquely capable of the requisite degree of intensified operation, large-scale implementation and genuine economies in energy and raw materials; the unique structure and character of natural lignocellulose composites makes enzymic hydrolysis inevitably slow, non-intensive, and non-economic in both raw materials and energy.

However it must also be stated that *at the time that project IND/80/003 was proposed, considered, and authorized this was a minority opinion and the general climate of belief was that these propositions were sound.* Moreover it must be added that in spite of the above objections to the

"cellulase scenario" the development of useful sources of cellulase enzymes remains a valid objective in itself because of the undoubtedly useful properties of such enzymes in a variety of specialist operations, to be carried out on a smaller but still economically significant scale. Moreover that part of the programme which relates to the pre-treatments which are necessary in order to make natural lignocellulose composites more susceptible to attack by cellulases has an intrinsic value since such pre-treatments themselves generate products which are valuable in their own right, for example as improved animal feed materials.

In those terms the project at NCL was well-conceived and in general well executed, and within those terms the success achieved was satisfactory. Moreover the decision eventually reached (by a tripartite review meeting) to wind down this part of the project after consolidating the results obtained up to that point was in this consultant's opinion entirely correct. This part of the project has therefore to be assessed within the above framework of considerations, and this line is followed here.

## Criticisms of the cellulose/cellulase project

First it must be admitted that the project suffered from the involvement of too varied a selection of external advisers, each of them certainly distinguished but few of them being sufficiently extensively involved with the project to ensure that their advice could be continuously tested and where necessary re-formulated in the light of actual project experience. As a result, some NCL efforts were un-necessarily diverted into fruitless trials of contradictory advice. **It is recommended that in future projects the selection of external advisers should be such as to involve the advisers in rather more than a single transient visit with no stake in the success or otherwise of the project as a whole.**

A second criticism of the project is that because of the internal structures of NCL the project was initially too closely confined to one technical section of the Laboratory; in particular, inputs from process engineering at an earlier stage could have ensured that some results were achieved more expeditiously and perhaps with more effective spin-off into transferrable technologies (for example, bagasse conversion into cattle feed by steam explosion would have been an accessible technology if steam explosion had been investigated as a pre-hydrolysis treatment from the start).

## Achievement of NCL in the cellulose/cellulase project area.

First, the record of publications arising from this project lists some 41 papers, all appearing in refereed international journals (to be reported in detail by NCL) whose effect is to establish the NCL as a centre of expertise in the specific field of cellulase production and properties (by no means confined to the more commonly-encountered types of cellulase). New types of cellulase - in particular the cellulase complex of *P. funiculosum*, have been brought into consideration internationally as a result of NCL work. Perhaps more significantly, the work on cellulase has given NCL a corresponding authority and expertise in the more general - and ultimately more important - field of microbial enzyme selection, production and improvement, and in the developing technologies of the cultivation

of enzyme-producing organisms and of very varied enzyme applications. This is an indirect but very considerable benefit for the country as a whole; the creation of indigenous enzyme technologies and expertise is an essential aspect of strategic biotechnology for any development programme.

**Second, the eventual reconsideration of this part of the project introduced project leaders to the importance of wider issues in deciding research strategies in a national context that relates research to real economic and social situations.**

Third the project introduced NCL staff to the problems of internal technology transfer, first in transferring enzyme production from microbiological research contexts into fermentation development and second in the elaboration of experience into the fully-documented process design study which NCL successfully completed. The completion of this design study in effect marks the successful termination of this part of the overall project.

Fourthly a substantial proportion of the career development and training for NCL personnel achieved under this programme was specifically linked to the work on cellulase systems.

ethanol biotech--nology

## ethanol fermentation technology

### Introduction

In the event this part of the overall project has become the most substantial and it is one in which the most significant and positive successes have been obtained.

It will be recalled that this part of the project was related on the one hand to the cellulase studies, insofar as the latter were to generate fermentable glucose for bio-conversion into ethanol, but was equally intended to be independent of this, since in the Indian context even greater economic importance attaches to improvement of the technology for bio-conversion of the molasses which are an inescapable by-product of the large Indian cane-sugar processing industries. The ethanol project was also related to an independently-conceived programme for the improvement of energy-requiring process operations, such as (in particular) distillation, by the development and application of heat-pump technology, and it is satisfactory to note that this latter programme has been a successful one and that the requisite final construction and demonstration of heat-pump-assisted ethanol distillation units has been virtually completed.

The ethanol fermentation studies were from the beginning directed towards the useful and valid objective of intensifying the fermentation step so that it can be carried out with lower costs in materials, investment, and labour for a given output. This was to be achieved by:

(1) the selection and adaptation of yeasts with intrinsically superior characteristics.

(2) intensification of the fermentation itself by operating under less adverse kinetic constraints and with higher concentrations of the yeast biocatalyst.
(3) - necessarily for the realisation of (2) - the development of bioreactors suitable for the intensified fermentation.

Such studies therefore have both a microbiological/physiological component and a bioreactor systems/operation component, together with a strong requirement for these superficially separate components to be developed in a fully-interactive way.

## Yeast selection

In the selection of improved yeasts the general objective of improved tolerance to the ethanol produced is combined, for specifically Indian circumstances, with the aim of improving tolerance to inorganic salts (which are at high concentration in most Indian molasses) and elevated fermentation temperatures (since temperature control is in most circumstances difficult if attempted at all). Unfortunately the combined inhibitory effects of ethanol, salts, and temperature are additive and limit the fermentation in two ways. Reduced performance in the yeast limits the efficiency with which the substrate sugar is converted into product ethanol (i.e. both *yield* and *conversion* are reduced). The reduced tolerance to ethanol caused by the other effects lowers the final concentration of ethanol that can be attained and so increases the financial and energy cost of prodct recovery.

From fermenting cane-juice by careful selection, the NCL group isolated two strains of *S. cerevisiae* with significantly improved salt and ethanol tolerance. In trials with molasses media (25% total solids) these strains demonstrated superior performance, reaching acceptable final ethanol levels in significantly reduced time and with good conversion efficiency. Though these strains were developed in the context of the search for good yeasts for immobilization and continuous operation (*see below*) strains of this kind should be of wider interest and they are available for testing in conventional systems. Equally the NCL team has shown itself capable of developing useful strains and this aspect should now be pursued routinely in collaboration with industrial operators who can put newly-developed strains into realistic tests prior to actual introduction. The extension of this part of the work to include some improvement in temperature tolerance is desirable particularly now that the team has a better idea of the *totality* of requirements for a good industrial strain, which include requirements arising at all stages of the process profile. Some of these requirements, such as flocculation characteristics of a particular type, will vary very considerably with the precise nature of the operators process system.

## Process intensification—general aspects

Apart from strain improvement the main routes to process intensification for the ethanol fermentation are (a) better use of existing installations by modified process management—better temperature control, yeast separation and recycle, fed-batch operation, etc; (b) redesigned processes for continuous operation. For a project of the present nature the first of these routes is not directly significant since it can only be imple-

mented by work actually in an industrial installation; the second has a significant research content and will in general follow two approaches, separately or in combination:

(i) removal of the inhibitory product ethanol during the fermentation, to provide better process kinetics while facilitating product recovery.

(ii)increasing the concentration of yeast bio-catalyst permanently in the reactor so as to increase the volumetric rate of the process.

Approach (i) is generally considered to involve the application of too high a level of process technology for economic application to a relatively low-value product such as ethanol and so in this project attention was correctly concentrated on (ii), a technology which depends on obtaining some means of either yeast retention or yeast recycle (these two being effectively equivalent in certain systems). Yeast retention was first pursued by the application of conventional cell immobilization techniques, and second by the application of an auto-immobilizing (permanently-flocculated) yeast strain.

## Yeast immobilization

The techniques of cell immobilization are increasingly important *per se* as a means of applying many different kinds of biocatalytic effect. Such techniques are essential for the development of so-called "second-generation" bioreactor systems, which is a cardinal objective for any serious biotechnology programme, and involve the development of competence in certain general procedures, the modification of those general procedures appropriately for particular systems, and the process-specific design, construction and operation of appropriately-scaled bioreactors.

In a series of papers directly arising from the ethanol project the NCL group have established precisely such capabilities, exploring different techniques for cell immobilization, devising reactors in which immobilizates could be tested and/or used, and establishing design and performance limits. This competence is directly relevant not only to the present project but more widely, extending to reactors in which other kinds of cells, or enzyme preparations, can be used for other kinds of biotransformation; this is an important outcome of the project because it is very desirable for there to be centres of competence in these far-reaching technioques and it is very logical that one such centre should be at the National Chemical Laboratory with its established reputation in conventional catalysis processes.

In the specific context of this project the NCL made progress in the development of immobilized-yeast ethanol reactors which compares favourably with work in other countries. This work is described in a significant series of research papers, and it is also embodied in 'in-house' know-how. As a result of this work, however, it became clear that a process of this kind would be very difficult to operate with Indian raw materials and was likely to require process parameters and plant that would not be economically viable. This is not a negative result; it is as important to establish the limitations of a technique as it is to appreciate its potentialities. The eventual decision to use the special advantages of an auto-immobilizing yeast *(see below)* was made correctly in the light of positive experience using other immobilization approaches.

### Auto-immobilizing yeast

Certain strains of yeast, cultivated under correct conditions, will themselves form particles with essentially similar properties to the composite biocatalyst particles produced by cell immobilization methods. Again, the technique is one of potentially wide application in the development of new bioreactor systems, with the specific advantages that:

(a) the particles do not require any special raw materials for their construction.
(b) they can in general be prepared *in situ* and so do not require any separate facility
(c) they are in general self-replacing, avoiding periodic shutdowns and in effect having indefinite life
(d) being entirely composed of biocatalyst, rather than compounded with some inert material, they allow far higher concentrations of catalyst to be attained within the reactor.

Where a suitable reactor can be applied, therefore, such catalysts offer major advantages.

The final phase of the NCL ethanol programme was therefore based on this approach and appears to have enjoyed considerable and rapid success, with improved collaboration between the process engineers and the microbiologists as an essential pre-requisite for this. Small-scale reactors have been constructed and operated for long periods with good results, the process management of these reactors has been refined, the design of suitable pilot-scale reactors has been completed and their construction and installation was at the time of the consultant visit well advanced with every prospect of early completion. Certain temporary problems connected with the concept of preparing the catalyst *in situ* rather than by a separate operation, and with the design of reactors which combine good mixing with effective yeast retention, have been overcome.
Detailed technical reporting of these results is to be found in the reports being prepared by NCL directly and no such account is attempted here; however it should be said that the preparation of these reports, and indeed the whole of this phase of the project, have been carried out in close and effective consultation with the UNIDO consultant.

**final results**

There is every prospect that a demonstration pilot installation, which will be coupled with the heat-pump-assisted distillation system, will be operational in 1988, and there are satisfactory indications of genuine industrial interest in taking-up the developed technology. Such interest is likely to extend beyond India, for example to Brazil, giving the NCL a real chance of a significant 'first'.

**Achievement of NCL in the ethanol project.**

The main achievements have already been noted, and can be summarized as comprising:

(1) establishing a centre of expertise in cell immobilization methods

(2) establishing specific experience and research publication in ethanol production using immobilized yeast and establishing the specific limitations of this approach

(3) successful development and imminent pilot-scale demonstration of working bioreactors suited to Indian substrates and conditions using auto-immobilizing yeast as biocatalyst.

An achievement which should follow from (3), and for which the foundations are already established, is the development of direct contact and collaboration with industry, from which the basis for successful technology transfer can be expected to develop.

In addition—

— the overall achievement in terms of research publication should be noted with a significant list of publications in international journals,

— in terms of training and career development the undoubted benefits are detailed in NCL's own reports,

—it should also be noted that such success has its own special benefits in facilitating the development of future collaborative and international programmes in other areas of biotechnology.

We should finally note the benefits in terms of improving the working collaboration between different disciplines and the overall management of interdisciplinary projects as represented by the different administrative sections of NCL. Poor contact and communication between these sections has at times been a serious problem within the project, and the overcoming of these obstacles is correspondingly a genuine and significant achievement.