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14877

July 1985
ENGLISH

India.

BIOSCIENCE AND ENGINEERING.

DP/IND/80/003

Technical Report*,
Mission from 15 to 27 January 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 J.D. Bu'Lock
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United Nations Industrial Development Organization
Vienna

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1. Assignment. To advise and assist staff of the National Chemical Laboratory, Pune, India, in relation to the designated project and in particular to their studies on conversion of cellulose into glucose and of glucose and molasses into ethanol.

2. Logistics and Itinerary.

There were no problems with either logistics or itinerary on this occasion. Communications with Vienna were greatly improved as compared with 1983/4 and the local travel and accommodation arrangements all functioned smoothly.

I travelled privately as far as Bombay some time before the assignment commenced, and my remaining itinerary was as follows:

January 14-15 Bombay stopover
January 15 Bombay-Pune (rail): initial discussions
January 15-27 incl. : on assignment
January 27: final discussions; Pune-Bombay (rail)
January 28: Bombay stopover
January 29: Bombay-Paris-Manchester (air).

The period January 15-27 was divided between individual discussions with section leaders and project personnel, group discussions with different sections of the project, general discussions with the assembled project team, and strategic discussions with the Director and Deputy Director of NCL. It was also possible to spend some time with Dr Karanth, who for much of the previous year had continued to play a major role in the project and who is now transferred to the Food Technology Research Institute in Mysore.

The principal NCL participants were:

Professor L. K. Doraiswamy (Director)
Dr R. A. Mashelkar (Deputy Director)
Dr M. C. Srinivasan
Dr J. Barnabas
Dr C. SivaRaman
Dr. Mrs H. SivaRaman
Dr R. B. Mitra
Dr G. R. Venkitakrishnan
Dr S. H. Iqbal
Dr A. C. Manchanda
Mr A. P. Pendse

but other persons, of all grades, were also most helpfully involved.

It is a particular pleasure to report the very warm welcome I was given at NCL and the very great efforts which were made by all concerned to ensure that my visit was a happy and comfortable one and to provide the best possible conditions for fruitful discussion and informed comment.

I should also like to note that it was clear throughout that a very high regard was given by NCL to the comments and reports that had been made about this project from other advisory visits, including my own, and that very real efforts had been made to follow up the suggestions and leads which those reports had provided.

3. General Progress of the Project.

If we review the project as a whole there is no doubt that a good deal of excellent research has been carried out, which must be accounted as successful in general terms. In a project of this nature it is inevitable that many lines of research have been opened whose eventual conclusions are of academic rather than technical interest; this is because until the research has been pursued to a certain stage its technical value remains quite unknown. In this sense, even the work that does not have a successful and direct technical outcome nevertheless makes a real technical contribution, because it delimits areas of technical interest more closely.

The general project area is one which has been widely canvassed as offering major development prospects; only by exploring it in some depth can the real limitations of those prospects be ascertained, and the practical directions of genuine economically-significant contributions laid down with greater accuracy.

In these terms the project has already qualified as successful. The aspects of the research which should be most fruitfully finalised are now clear. However, regard must be now paid to the need to bring the present project to its concluding stages in the foreseeable future. I am satisfied that it will be possible to do this with very significant progress towards industrial applications having been made, provided these conclusions are now given internal priority.

This means that progress in some particular aspects needs to be reviewed with the aim of bringing them into an integrated whole. Immediate priorities need to be assessed in that context, and this leads to some changes of emphasis from the priorities that would be assigned in an "open-ended" situation. Some solutions that are known to be sub-optimal have nevertheless to be accepted for the shorter-term purposes. Instances of this are made clear in subsequent sections of this report.

At the same time the project itself, reviewed in a wider context, forms a significant base for longer-term considerations of strategic priorities for biotechnology research at NCL, and this aspect is considered in a separate section.

4. Cellulase production and utilisation.

This programme has benefited from recent technical reports from Dr Erikson and, in particular, Dr Mandels. However their recommendations must now be taken in the context of a programme which needs narrowing-down towards finalisation, and the temptation to introduce new directions of "academic" interest, which would be entirely acceptable in a different context, must be resisted.

Immediate steps should be taken to determine a procedure for producing the P. funiculosum enzyme in a way which is practical, repeatable, and capable of being run in the largest available equipment, and which gives the highest available cellulase level.

The enzyme level should be measured in standard units, and its attainment in a reasonable time is also important. The substrate for enzyme production should be optimised for enzyme level; there has been some past confusion as a result of accepting uncritically the observation that the eventual level of enzyme is determined by the biomass concentration reached. This is only true when comparing otherwise equivalent conditions. In practice it is more important to ensure that the biomass is giving maximum specific enzyme production; only within that pre-condition is a higher concentration of fungal biomass useful. For high specific enzyme production the substrate is, as pointed out by Mandels, one which offers a "challenge" to the organism. Such a substrate is not, in general, one which - compared with other substrates - gives highest biomass.

Once procedures have been decided it will be important to make a dispassionate comparison of what has been achieved with P. funiculosum with what is possible with T. reesei. The enzyme levels achieved should be compared at a useful substrate conversion level. For the purpose of comparison the T. reesei preparation should be combined with additional cellobiase, for which P. funiculosum is already established as an excellent source.

While we tend to agree that for real commercialisation the enzymic hydrolysis will probably use a steam-exploded or similarly physically pre-treated natural feedstock, we also accept that such a feedstock is not immediately available locally for meaningful study, and the decision to continue this part of the work using alkali-treated feedstock is correct.

The comparison with T. reesei is for the purpose of deciding if it is worthwhile continuing work with P. funiculosum as a cellulase source. For this purpose it is sufficient to establish that P. funiculosum wild-type under the best locally practical conditions is better than early T. reesei strains under comparable conditions; i.e. the aim is to establish whether the alternative organism shows genuine prospects. Such a comparison would lead to a clear decision as to whether or not to persist with strain development and fermentation process improvement.

Applications of "molecular biology" to this immediate problem of strain development are unlikely to generate practically-useful results ina reasonable time.

Realistic hydrolysis studies (to a significant % conversion) should then be carried out, using either the best available P.funiculosum enzyme or the commercially available T. reesei enzyme supplemented with P. funiculosum cellobiase. The hydrolysis studies should include studies of how to maintain the required degree of asepsis, and how to recover the product and as much as possible of the enzymes. Only studies of this kind will enable a

realistic assessment of the prospects for an enzyme hydrolysis process to be assessed, and compared with the existing and anticipated data for other procedures for lignocellulose exploitation.

Meanwhile practical (scaled-up) technical procedures for the production, recovery, and applications of P. funiculosum cellobiase should be developed in any case; the availability of this enzyme would be an immediate result of commercial interest. As already indicated, this will apply equally to this organism's cellulase, if the comparison outlined already turns out positive.

This is because, irrespective of the economics and practicability of enzyme hydrolysis as a major exploitation route for lignocellulose, the availability of good hydrolytic enzymes for a great variety of process uses (in food and agro-industry) is itself a very valid objective, and contributions of this kind would be a directly valuable result of the excellent work so far carried out.

5. Yeast strain development.

Work under this heading is an important aspect of the overall project, since it offers the possibility of results which can be directly introduced into existing Indian technology quite irrespective of the project as a whole. This means defining the objectives of strain development as a search for better temperature tolerance and better salt tolerance without sacrificing rate, yield, or physical characteristics used in existing processes. It also means strengthening the working relationship with industrial operators.

Improvements of this kind would benefit existing industry even though this is at present running below capacity. In these circumstances product yield from feedstock is quantitatively more important than plant productivity.

With similar objectives the promising work on added agents such as skim milk powder which appear to protect the yeasts and to improve their characteristics should also be continued right up to plant-scale trials.

6. Immobilized yeast reactors.

This aspect of the work received the most particular attention during my visit; we were able to discuss it not only in abstract but by direct reference to a working system. This part of the work has sen both successes and set-backs, but its overall progress is very clear. To some extent it is perhaps in need of more decisive central management, particularly as the time for finalised decisions approaches. At such times, peripheral studies which at earlier stages were really valuable can become un-neccessary diversions of central attention.

The laboratory scale is the correct one at which to try out and implement decisions regarding the method of immobilization; the current conclusions from this work appear to be essentially correct. It is important, however, always to carry out the laboratory scale work in ways which are capable of being scaled-up with as little extra difficulty as possible, and equally, always to assess the laboratory results in this light.

As regards work on the "large-laboratory scale" (i.e. the 10-litre reactor system), we had several very useful discussions not only of the work done at Pune but about what could be learnt of pilot-scale developments in other countries. We discussed specific operating difficulties under the headings of:

yeast culture
amount of yeast to be immobilized
immobilization protocols
post-immobilization protocols
reactor loading and operation.

A general recommendation was to very thoroughly minimise delays and hold-ups in the overall sequence of setting up a working reactor system.

Specific recommendations, agreed after exhaustive discussions, were as follows:

Preparation of yeast. The immobilizate is to be prepared with an "inoculum density" of yeast and the immobilized preparation then incubated under growth conditions to build up a "working density" of yeast in the particles. Ths gives a more efficient immobilizate, especially if larger particles are used, and reduces the amount of yeast needed initially. In technical operation the advantages will be similar.

The inoculum yeast is to be grown in a "clean" medium and prepared for immobilization with the minimum number of steps. If the immobilizate can be prepared in the reactor itself it will be advantageous.

Immobilization. The inoculum yeast is to be co-immobilized with protective agents (sterol, unsaturated lipid, skim milk) and the beads prepared for good mechanical properties.

All procedures should if possible be "clean" rather than sterile. Conditions to reduce contamination include presence of ethanol throughout, possible use of antibacterials, low pH, etc.

To reduce gas hold-up in the reactor it may be helpful to prepare rather larger immobilizate particles.

Reactor operation (10-litre). To assess the operation of the simple upflow 10-litre reactor it is desirable to establish (by successive experiments that go beyond the optimum each time) the maximum substrate flow rate that will give 6-7%w/w ethanol at 95% of the maximum practical conversion, at a given loading density of particles, and to increase that loading density stepwise from one experiment to the next until mechanical failure (usually through gas hold-up) of the single stage reactor is seen. Failure is when the particle bed is no longer in a "mostly fluidized" state.

Because the 10-litre reactor is to be viewed either as a model for a single-stage process or as a model for one stage of a two- or three-stage process, its characteristics should also be determined, by an outline series of experiments as above, with 50% or 35% conversion [both of unprocesed substrate and of part-processed (ethanol-containing) substrate] as the target. It may be convenient to construct and run a 2-stage reactor at this stage.

At a later date the operation of the reactor using non-molasses substrates (cane juice, starch hydrolyzate) should be assessed at least in a preliminary manner.

Reactor design (100-litre). In the light of studies on the 10-litre scale it will be possible to advance design details for the 100-litre reactor. The shell of this reactor has already been built, and is basically satisfactory, but further construction should be halted until a clearer picture of the design requirements (1 or several stages, single or multi-compartment, separate or collective gas escape, etc) is obtained from studies on the 10-litre scale.

Relation to other projects. Investigations of the suitability of cellulose hydrolyzates for ethanol fermentation by these techniques should not be given high priority.

The present state of the heat-pump project at Pune seems to be ahead of the ethanol reactor project; however the heat-pump project can presumably be progressed without the ethanol reactor since it is also applicable to existing ethanol technology and compatible with existing distillery installations.

7. Post-finalization: A Strategy for NCL Biotechnology.

There is no reason to suppose that the NCL project will not result in the development of a workable technology for molasses alcohol. However the industrial take-up of that technology will depend on many factors which are outside the developer's control.

For the immediate future the situation in India is foreseeably that:

- any expansion of sugar cane plantings will be irrigation-dependent and geared to crystal sugar production and will not be very great; consequently the industry will continue to generate an essentially similar molasses situation.

- the molasses situation is dominated by internal taxation policies and the possibility of selling surplus molasses abroad with minimal processing.

- ethanol production from molasses will be geared to home markets, in which potable alcohol generates higher profits for the distiller and distributor and also provides a traditional tax base.

- at the present time the market for industrial ethanol is, if anything, declining,

- greater production of ethanol for industrial use will mainly be conditional on political initiatives.

- the present fermentation and distillery capacity is under-used so that only a vary major increase in industrial ethanol production would generate opportunities for new plant.

In these circumstances the opportunities for direct adoption of NCL's molasses-based ethanol technology are real but limited, and should not be anticipated on any short time scale. This is why I have emphasized, in earlier sections of this report, certain "lateral" aspects of the programme that are capable of more immediate transfer into industry; for example, use of P. funiculosum as a source of cellobiase; screening for more efficient yeasts; etc.

As regards the lignocellulose technology being developed at NCL I have to express similar reservations, admitting that these run contrary to much international advocacy by more committed parties.

Existing lignocellulosic "waste" in India is to a large extent already fully-utilized, as a fibre, as an animal feed, as construction material for rural habitations, and (particularly) as a rural fuel. Under these circumstances there is undoubted scope - particularly under any central programme for "waste lands" - for increasing lignocellulose production, without generating any corresponding increase in the availability of lignocellulose for hydrolysis technologies of any kind. However, as before, this does not mean that technology of cellulase and related enzymes should be wasted. There are a growing number of uses or possible uses for such enzymes whose adoption will depend on their availability; these uses are precisely in a sector of the Indian economy which is expanding, namely the small- or middle-scale processing of primary agricultural crops for secondary derived food products. These uses will demand a developed technical capability that can operate to adequate standards, and NCL's established expertise here will be a major asset that should be properly deployed.

The situation as regards starch crops is quite different.

Here there is only limited scope for increased production of primary food items, but major scope for the production of starch for industrial exploitation; a high proportion of the starch thus exploited will end up in food items, but of a derived nature and with considerable added value. Given the rather different agronomic requirements, increased starch production could play a major part in any "waste lands" project, and this in turn will generate a major need for starch enzyme technology. At the present time India has some of the requisite technology but is mainly dependent on imported know-how and (particularly) enzymes, especially of the higher grades and for the more sophisticated processes. I see this as the main future origin of industrially-useful "biomass" in India and consequently as the main future need for indigenous technical capability. Moreover I see outlets for NCL's existing committments in these same directions. The ethanol work will be even more readily applicable to starch hydrolyzates. The basic expertise in selection of micro-organisms, optimisation and enzyme production and use, has all been reinforced by the present programme and should increasingly be brought to bear in these directions as that programme runs into its final stages. This is the way to derive maximum benefit from all that has been done so far.

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