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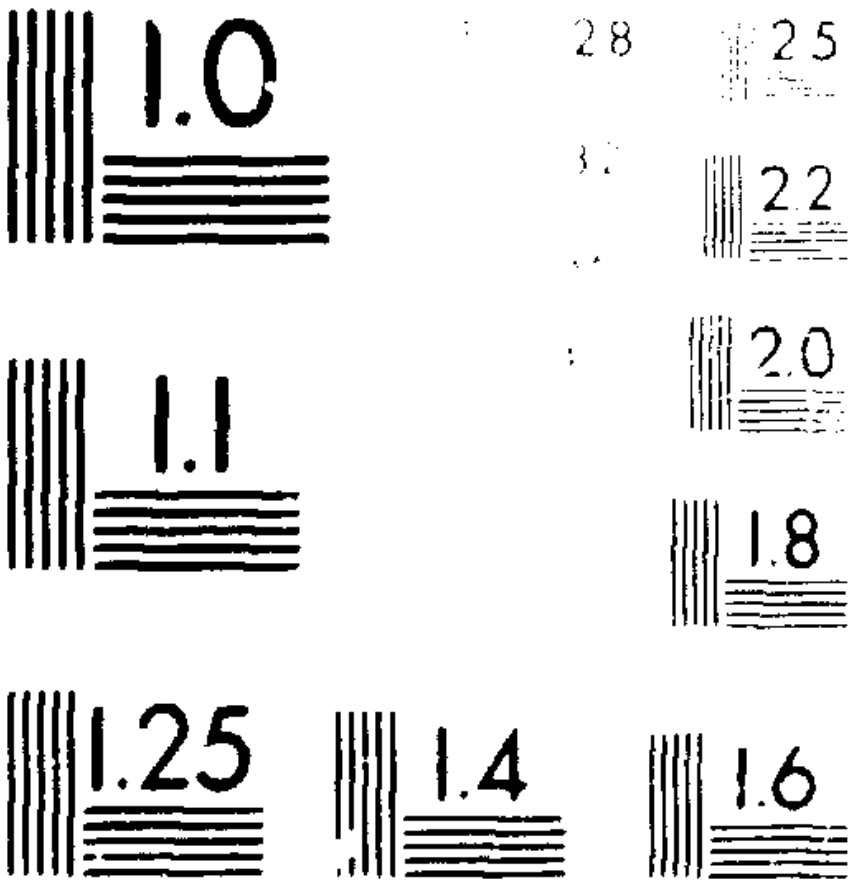

MICROCOPY RESOLUTION TEST CHART

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INDIA

Technical Report *

Mission 23 November to 6 December 1984

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Based on the work of Karl-Erik Eriksson,
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Vienna

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1. PURPOSE OF MISSION

The purpose of this mission has been to advise on the following activities at the National Chemical Laboratory (NCL), Poona, India.

(1) Advice on the development of biotechnology for utilization of lignocellulosics for microbial biomass production (MBP).

(2) Advice on the process development for production of ethanol from lignocellulosic materials.

(3) Advice on the process development for production of ethanol from molasses.

2. Utilization of lignocellulosics for the production of microbial protein (animal feed) has been studied using a strain of Penicillium janthinellum, isolated from soil at the NCL campus. This organism has been used for the conversion of rice straw to microbial biomass. The fungus is reported to metabolize alkali pretreated straw yielding a biomass containing 15 to 20% protein. However the activities at NCL have not been particularly focussed on this development although a few papers have been published on this subject.

At the Swedish Forest Products Research Laboratory (STFI) a process for production of microbial protein from lignocellulosics has been developed based on the white-rot fungus Sporotrichum pulverulentum. It has been found that with glucose as substrate a fungal biomass containing 42% protein can be produced. However the protein content in the mycelium decreases with increased complexicity of the substrate. The reason is that the more complex the substrate the more enzymes (proteins) have to be

secreted into the medium to digest it. Particularly with a substrate of high lignin content and with a high content of crystalline cellulose you will face difficulties. It can be concluded from our development that although it is possible to produce a biomass with a reasonably high protein content in this way it has not turned out to be economically feasible. The reason is the competition from low priced soya protein.

To develop a more economically feasible process, the white-rot fungus S. pulverulentum was utilized for water purification particularly of white water systemsof board mills and newsprint pulp and paper mills. A part stream of circulating white water was transferred to a fermenter. In this fermenter degradation of organic materials such as water soluble sugars, extractives and lignin degradation products was carried out by the fungus. Microbial biomass could in this way be produced from a carbon source having a negative value. As a result a process allowing an almost complete abatement of the effluent problems in the paper mill system was obtained. The produced fungal biomass was evaluated as feed in animal feeding trials which went on for two years at the Agriculture University of Uppsala, Sweden. The fungus was found to be a suitable feed for ruminants but not so feasible for monogastric animals since the fungal cell wall is difficult to digest. The produced fungal biomass was as an alternative to animal feed, added to the produced newsprint paper for increased paper production. A paper containing 1.5% (w/w) fungal biomass showed no differences in quality from a paper without fungus.

Based on the above it is suggested that one scientist from NCL comes to STFI (when space can be made available) to compare P. janthinellum and S. pulverulentum for the purpose of microbial feed production from lignocellulosics. It is suggested that steam exploded wheat straw is used as substrate in these comparison studies.

The reason why P. janthinellum should be compared with S. pulverulentum is that one of the most costly parts in the development of a process for microbial biomass production is the feeding trials that must be carried out until the microbial protein can be accepted as feed. Such feeding trials have already been carried out with the S. pulverulentum biomass. The process technique developed for this fungus can also easily be adapted to the lignocellulosic materials available in India.

The possibility to produce an easily digestible animal feed by upgrading lignocellulosic materials now exists. STFI has developed a process for delignification of wood and other lignocellulosics based on a white-rot fungus and cellulase-less mutants (Cel^-) obtained from it. The Cel^- mutants cannot degrade cellulose but degrade lignin substantially. This degradation can be carried out at the cost of part of the hemicellulose content. White-rot fungi need sugar to sustain growth and for the production of H_2O_2 which is absolutely essential for lignin degradation by these organisms to take place. The development at STFI means that in 2-3 weeks treatment as much as 25% of the lignin can be removed in straw, sugarcane bagasse, hard wood chips, etc. The delignification improves the digestibility of agricultural wastes by ruminants. A process of this kind would probably be of great value for India if it can be made to

function with low level technology. The technique is now developed in Sweden to obtain a technique which is simple enough to be used directly by the farmer. If this process development is successful it may be that the technique can be made available (licensing) to NCL scientists and thus developed also for the specific agricultural wastes which are available in India.

3. PRODUCTION OF ETHANOL FROM LIGNOCELLULOSICS (PROCESS DEVELOPMENT)

Basic work for conversion of lignocellulosics to glucose and ethanol has been in progress at NCL for about a decade. In a screening programme undertaken to identify microbial strains particularly suited for production of extracellular cellulolytic enzymes, two fungi, viz. Penicillium funiculosum and Sclerotium rolfsii were selected for further investigation. Basic work carried out with these two fungi have given rise to many published papers which generally have a good scientific standard. Recently the work has been focussed on an increased production of cellulolytic enzymes from P. funiculosum. This work holds some promise particularly since this fungus is a high producer of β-glucosidase. I find, however, that the activities in this programme(to obtain a technical process for ethanol production from lignocellulosics) have been too scattered and have not been focussed enough on the given target for a technical process, or at least an economic evaluation of the feasibility of such a process. In view of this I therefore suggest that the following investigations and developments are undertaken:

Most of the efforts presently undertaken at NCL for enzyme production are focussed on the use of P. funiculosum with sodium hydroxide pretreated cellulose as substrate. It is

clear to me that pretreated pure cellulose is too expensive even to be considered for cellulase production. My suggestion is therefore that a substrate for enzyme production is chosen which relates to what is available in India. This substrate should be one of the following:

(i) rice straw (production per annum 59.2 million ton),

(ii) sugarcane bagasse (production per annum 52.1 million ton),

(iii) rice husks (production per annum 18 million ton) or cotton stalks (production per annum 12 million ton).

(iv) Where hard wood species are available and not used for any other purpose, such a substrate would also be used.

It seems desirable that the enzyme production takes place on the same substrate which will later be used for saccharification and sugar production purposes. However, since wheat bran has proven to be a useful substrate for enzyme production, this carbon source should also be considered.

The pretreatment technique used at NCL is mainly chemical, but sodium hydroxide. This is, however, an expensive technique and not a very effective one. My suggestion for pretreatment is therefore the IOTECH process developed in Canada. This is a steam explosion process where the substrate is heated during 50 seconds to between 234 and 238°C. The pressure is then suddenly released which makes the lignocelluiosic material explode and form a fine powder. If hard woods or grass species are used, the hemicelluloses are easily extracted with water and the lignin with either sodium hydroxide or ethanol. We have employed the IOTECH process for pretreatment of wheat straw which is the substrate used in saccharification studies at STFI. Yields as

high as 90% of available sugars have thereby been reached. The IOTECH process seems to be an economical way for pretreatment since it has a low energy consumption. My suggestion to UNIDO is that money is made available for purchase of the necessary equipment to allow construction of the IOTECH machinery at NCL. I have personally good contacts with Mr. DeLong in Canada who is the inventor of the process. An installation of IOTECH machinary is already made at Centre Technique Papier (CTP) in Grenoble, France. It may be that pretreatment of a suitable substrate can be made at CTP until the equipment at NCL has been realised. The IOTECH process for pretreatment of lignocellulosics would be useful for NCL in many ways such as development of technique for utilization of lignin as a polymer or for production of basic chemicals etc.

As pointed out above, the production of cellulolytic enzymes are presently studied at NCL only utilizing the P. funiculosum strain. My suggestion would be that all the work is not to be focussed only on this organism. Several very potent cellulase producing strains are now available. These strains are the results of mutation and selection work in various laboratories particularly in the United States and Finland. My suggestion therefore is that five of the best cellulase producers available, including NCL P. funiculosum strain, are chosen and brought to NCL for comparison of their enzyme production. This production should be carried out either on the substrate chosen for the saccharification work (see above) or on wheat bran. The selection of the best strain can be done by studies of enzyme production in shake flasks. When these studies are completed the best strain for enzyme production is

chosen and its production optimized in studies at fermenter scale. It seems likely that batch fermentation with repeated addition of substrate is the best available technique. The enzyme production per unit time is of course the most important factor. In particular the amount of extracellular protein and the protein/cellulase ratio should be studied to be able to make a judgement of the potential for a possible improvement. This is a particularly important point for estimation of the economy and for estimation and judgement of where to insert your development efforts; should it be in the development of new strains; development of hybrid organisms by protoplast fusion; transfer of genomes for coding for cellulases from one organism to another etc. or to obtain better strains by some other technique.

A considerable input of work has already been done at NCL in the saccharification studies. These studies have been carried out with many substrates and with several different pretreatment techniques. As said above, I suggest that one substrate is chosen and that the pretreatment technique is the IOTECH process. The development of an economical saccharification process based on enzymes is particularly important and I suggest that this part is given substantial resources especially by assigning biochemical engineers to this work. Sugar yields and reutilization of enzymes are particularly important points in this context. Economic evaluations in Sweden have shown that enzyme hydrolysis of lignocellulosics will not be competitive with acid hydrolysis unless enzyme reutilization can be made as high as almost 95%. The goal for the development of the saccharification process should therefore be set at 90% yield of sugar and 95% reutilization of enzyme. It is important to remember that even if most of the

polysaccharides are solubilized by the enzymes, remaining lignin does adsorb the enzymes. This adsorbtion seems to be by hydrophobic interaction. Recirculation of the solid lignin-rich and also enzyme rich residue should be considered as well as elution processes. The pretreated substrate is saccharified by repeated addition of substrate until a sugar concentration is reached which is competitively inhibitory to the enzymes. At this stage the enzymes must be separated from the sugar which can be done by countercurrent technique or by membrane technique. Much discussion with the NCL scientists has taken place on this point and we seem now to agree on the best way optimize a process for saccharification.

In addition to enzymic hydrolysis the alternative, acide hydrolysis combined with a process for complete monosugar production by matrix bound β-glucosidases should be studied and the economy of the two processes compared. It may be that the P. funiculosum β-glucosidases are ideal for this purpose but also β-glucosidases from other sources (Aspergillus niger) should be investigated.

Besides focussing on the direct development of a process for ethanol from lignocellulosics I would suggest the following activities, at NCL, which strongly relates to such a process, to take place.

(a) Improvement of cellulase production in the P. funiculosum strain and particularly to study:
Cellulase production on the chosen substrate in fermenter scale by wild-type and already obtained mutants;
Selection of better strains from monospores cultures;
Production of new mutants from the best strain by continued mutation work;
Protein profiles of extracellular enzymes from wild-type and mutants by isoelectric focussing and technique.

(b) Protoplast fusion and gene transfer work:

It seems essential that NCL scientists develop competence in the molecular biology field of biotechnology. However, in spite of considerable discussions no agreement could be reached concerning the area on which to focus this work. Fields such as: identification and characterization of cellulase genes from Cellulomonas, studies of Zymomonas hybrids to which cellulase genes from Cellulomonas have been transferred, complimentation of genes in Sacchromyces cerevisiae to allow conversion of pentoses to ethanol, etc., were suggested.

(c) Pentose fermentation:

Production of ethanol from lignocellulosics in an economically feasible way must allow fermentation of pentoses which normally make up for 25% of the weight of agricultural wastes and hardwood. The following studies are therefore recommended at NCL:

Evaluate ethanol productivity by various available pentose fermenting strains and focus the work on the best. Its productivity should then be developed even further.

I strongly feel that this work should also involve the Neurospora crassa strain which has several virtues, i.e., it produces the enzymes necessary for polysaccharide conversion to monosugars under aerobic conditions and ferments these sugars, both hexoses and pentoses, to ethanol under anaerobic conditions.

4. PRODUCTIVITY OF ETHANOL FROM MOLASSES (PROCESS DEVELOPMENT)

This project is not within my general field of expertise. I feel, however, that the process development is in the hands of

competent people and believe strongly in the possibilities they foresee. I have gone through the reports of earlier consultants and agree fully with the recommendations given by Dr. Bu'Lock. This is particularly so concerning his given recommendations for upscaling.

I would in addition to this give the advice that the Biostil process, developed by the Alfa-Laval Company in Sweden is looked into as well as the so called Anamet process (anerobic fermentation with production of methane). The Biostil process is aimed for distillation of ethanol and the Anamet process for purification of waste waters containing organic substances whereby methane is produced. I will try to collect detailed information on these two processes and pass it over to the NCL scientists.

5. GENERAL COMMENTS

This report has been formulated after incissive discussions with individual scientists, the existing small groups of scientists designed for the different studies, the whole group of the biochemists, microbiologists and biotechnologists as well as with the Deputy Director of NCL and his close associates.

Two lectures have been presented by the consultant namely:

(i) Microbial delignification of lignocellulosic materials.

(ii) Utilization of polysaccharides in lignocellulosic materials for ethanol production.

I particularly acknowledge the willingness of participation in discussions and other activities shown by the NCL scientists. The overwhelming hospitality shown to me at all levels of the NCL staff is highly appreciated.