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JUTE RESEARCH AND DEVELOPMENT

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INDIA

Technical report: Biosoftening of Jute
(First mission)*

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 B. J. B. Wood, expert in solid substrate fermentation

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* This document has not been edited.

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## ABSTRACT

Jute Research and Development: Biosoftening of Jute. DP/IND/86/037/11-02/J13102. This Report refers to the first visit to the Indian Jute Industries Research Association, Calcutta, By Dr Brian J.B. Wood. The processes involved in the manufacture of jute thread and cloth are briefly outlined and the uses for enzymes in upgrading the jute fibre and in improving the tamarind kernel powder used for sizing are discussed. The visit lasted for two weeks including a journey to Mysore to discuss the project with the people who designed the enzyme production unit (koji plant). Comments on and constructive criticism of the design for the proposed koji plant are contained in an Appendix. Other Appendices report on the visit to Mysore and contain suggestions for future research. My next visit to Calcutta should be in September 1990 to assist in commissioning and operating the first runs in the koji plant. I am trying to obtain mould strains which produce an abundance of pectinase for improvement of the jute upgrading treatment and also for investigation in connection with proposals to mechanically strip the bark and bast from unretted jute and free the fibres with enzymes.

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# I. INTRODUCTION

## A. Nature of Project

The use of solid substrate fermentation of a mould to produce enzymes for the jute industry has been developed and shown to be effective in trials at working mills. The present programme entails the construction of a small factory to produce the enzymes on a commercial scale.

## B. Uses of Enzymes

The extracted enzymes are used in two ways in the jute mills.

### 1. Upgrading Tamarind Kernel Powder (TKP)

TKP is used as a size in jute mills. Treatment with the enzyme extract makes it easier to bring the TKP suspension in water to readiness for use and removes the need for boiling it, so saving time and heat. The enzyme-modified suspension's properties as a size are superior to those of the conventional preparation.

### 2. Upgrading Jute

Lower grades of jute have "root ends", which are incompletely separated bundles of fibres. The dispersion of these roots during preliminary treatment of newly received jute at the mill is greatly increased by treatment with the enzymes.

## C. Nature and uses of Tamarind Kernel Powder

TKP is produced by roasting Tamarind seeds, dehulling them and grinding the resulting kernels to a powder which has many uses as a thickener, particularly for foods. It replaced starch as a size for jute during the 1939-45 war, and has continued to be used so to the present. In the mills it is still referred to as "starch". Sizing makes fibres more supple and easy to handle in the spinning and weaving processes and also retains some moisture in the thread, which is furthermore coated, smoothed and given a certain amount of bulk by the size. When TKP is dispersed in water two classes of substances which it contains are important in jute sizing;-

1. The Complex Beta-Glucan Polysaccharide; this gives the paste

viscosity and coats the jute fibres, so discharging the functions for a size listed above.

2. The protein; this glues fibres together, improving the strength and continuity of the thread and reducing the frequency of breakages during weaving. Attempts to duplicate this function with other proteins such as gelatine have apparently been unsuccessful, and it is suggested that the proteins present in TKP have some special property which confers this useful function. There are four classes of proteins present in TKP. So far as I can determine it is not known which protein(s) or combination thereof has the special ability to glue the jute fibres together. In jute mills TKP paste is normally prepared by suspending TKP in the appropriate amount of water, then steam heating the suspension to maintain it at 100% until it is judged to have reached the correct state for use. In this Report that method is referred to as the "conventional process" to distinguish it from the "enzyme process". The conventional process is time-consuming, uses a lot of expensive steam and results in a product of variable quality.

## D. Outline of Jute Processing

The jute plant is cut down near the ground and "retted" by immersion in water until the outer fibres and bark separate from the woody core or "stick". The fibrous material is washed, dried and sent to the mill. There it is finally graded and mixed to give the desired blend, then put through a machine which stretches it and impregnates it with water and oil to soften it. Next it is piled in a covered heap where it ferments for a short period, being attacked first by aerobic, then by anaerobic bacteria (the latter depending on time and conditions). Then it is passed through a series of machines which gradually convert it to thread, meanwhile removing bark and other unwanted material. The thread is sized ("starched") woven, finished and baled.

## E. Outline of Enzyme Production Process

The selected mould is propagated from pure culture and inoculated onto sterile, moist wheat bran. This is incubated for several days at 30° in shallow trays to permit mould to first grow, then sporulate profusely. This preparation is carefully dried at low temperature and

is used as an inoculum for enzyme production, which is also done on moistened, sterile wheat bran, using 10% w/w of the spore culture as an inoculum. Bran is mixed with water in the Ribbon Mixer and spread into trays, which are sterilised in the autoclave. Sterile bran is tipped into the carefully cleaned Ribbon Mixer and mixed with the inoculum, then discharged back into the trays, levelled and loaded into the incubator. At maximum enzyme yield (approximately 2 days' incubation) the trays are transferred to the drying chamber and the bran is dried at low temperature (about 50°C). Dry mouldy bran is packed into 20kg sacks (contents of about 20 trays)for despatch to the jute mills. Alternatively mouldy bran (fresh or dried) is extracted with water on a countercurrent system to yield a concentrated extract which is treated with toluene to inhibit microbial growth, packed into containers and stored in a cold room until despatch to the mills.
Enzymes present in the koji and extract which are important in jute processing are pectinases, hemi-cellulases and beta-glucanases, while for TKP paste improvement the proteinases and beta-glucanases are the important ones.

F. Applications and Advantages of Enzymes in Jute Processing

1. TKP Paste

TKP is dispersed in water as before, aqueous extract of koji is added and the mixture is stirred and maintained at 70°C. Viscosity of the paste decreases due to partial hydrolysis of the complex beta-glucanase gum and the amount of protein soluble in water increases due to partial hydrolyses of insoluble protein. Thus there is considerable saving on heat as the prolonged boiling used in conventional TKP paste preparation is not required. When in use, the reduced viscosity of the TKP paste aids penetration of the jute fibres, so increasing the effectiveness of the sizing. The improved penetration and the increased amount of protein in solution enhances the glueing together of fibres by the protein. This gives a stronger thread, less liable to breakage. Mills using the enzyme-treated TKP paste report around 3% increase in weaving productivity over operation with conventional TKP.

2. Jute Processing

(a) Dispersing Root Ends

Root ends result from incomplete retting of the thick fibre bundles near the base of the plant and their presence downgrades the jute and leads to problems in its subsequent processing. If the moistened, stretched raw jute is heaped with the root ends on the outside of the heap and then enzyme solution is applied before covering the heap, the enzymes attack and hydrolyse the hemi-celluloses, beta-glucans and pectins which are binding the fibres into root ends. This attack also seems to promote and assist bacterial growth around (and hence attack on) the root ends. Consequently when the material is subsequently processed into thread the yield and quality are improved and the overall effect is to upgrade the treated jute.

(b) Processing without Retting

Inevitably retting, being an uncontrolled process depending on indigenous bacteria, carries a risk of loss of fibre quality; it also needs a lot of water and is very polluting. Consequently there is an interest in mechanically stripping the bark and fibre from the stick immediately upon harvest, then freeing the fibres from other plant material with enzymes. It is thought that the koji extract, particularly if supplemented with extra pectinase, could accomplish this.

## II. VISITS WHICH I MADE

### A. Jute Mill

I was shown around a Government Jute Mill in Calcutta. The processing of jute was most thoroughly explained to me and I was shown every stage from receipt of raw jut to packaging finished material.

### B. Mysore

At IJIRA's expense Dr B.L. Ghosh and I flew to Bangalore and thence to Mysore where we visited the Central Food Technological Research Institute to meet the personnel responsible for the proposed design of the koji factory (see Appendix). We had a most thorough discussion of the design and of the plant's operation. In particular, I had been very puzzled at the reports that solid trays gave mould growth no different from that in trays with air-holes bored into the base, but inspection of their trays showed that the holes were too few and too small to affect oxygen supply to the koji, and I stressed the need for

as much air access as possible, up to the use of mesh or expanded metal tray bases. As a compromise they are to increase the size and number of holes drilled in the base of the trays, although I continue to favour a more radical approach to the problem. Apart from this there was general agreement on the design and operation of the koji plant. I have made detailed comments on their design report in Appendix A.

## III. RECOMMENDATIONS

### 1. Design of Enzyme Production Plant

In addition to the comments on the CFTRI report describing the proposed koji plant (Appendix A) I have noted some changes in the location of equipment and the designated functions of areas in the koji plant on the copy of the plans thereof held by IJIRA. These proposals were made after the most thorough discussions with Dr B L Ghosh and Mr R Atkinson.

### 2. My Next Visit to Calcutta

It was agreed that this must be to assist in getting the koji plant into production. I have to go to a meeting in Holland 17th to 21st September, 1990 and propose to travel on from there for a period of one month In India including a visit to CFTRI Mysore.

### 3. Future Developments

I have requested a culture of a mould which produces a a high level of pectinase from Poland. I shall investigate ways of improving the design of the koji trays. If the market for enzymes outwith the jute industry develops as I think that is possible, there may EVENTUALLY be a need to go to more intensive enzyme production using aerated deep troughs, but it is ESSENTIAL that the present proposals for tray koji production be fully implemented first and that there be NO dilution of effort through premature exploration of alternative technologies.

Appendix A

DESIGN OF KOJI FACTORY

Comments on the "Detailed Project Report on Establishment of Solid State Fermentation Culture Plant for Production of Enzyme Complex for Use in Jute Industry" FOR Indian Jute Industries Research Association, Calcutta, BY Central Food Technological Research Institute Mysore (July, 1988).

1) Table 1.1 — The list of items only adds up to 62.9% that is to say 37.1% of the bran components are missing from the list.

2) P6, para 3 — It is recognised that shortage of bran in the Western diet is closely allied to the prevalence of 'Western' diseases of the intestinal tract such as diverticulosis. It is also suggested that such fibre reduces re-absorption of sterols from the digestive tract. Finally, there is increasing evidence that regular consumption of fibre leads to beneficial changes in flora of the lower intestine, principally an increase in bacteria which digest some part of the fibre and metabolise the products anaerobically yielding acids which both lower the pH of the intestine and are absorbed by the body and metabolised for energy. Consequently, there is a market for high quality bran as a component of human foods in the West.

3) P12, last line — Formaldehyde is no longer acceptable because of the hazard to workers. Use a commercial biocide formulated for cleaning.

4) Moisture content of wheat bran — 100g bran has 8.8g water and 91.2g solids. Add 50ml water and this gives

| | | |
|---|---|---|
| Solids | 91.2g | 30.4 x 2 = 60.8% solids |
| Water | 58.8g | 19.6 x 2 = 39.2% water |

For successful fermentation a water content of 41.5% is favoured for wheat bran in SSF.

5) Koji room — Should be set to at least 95% relative humidity. 13

6) P14-15 — Description and diagram do not agree with each other.

7) P23, Enzyme culture for assay — How is this clarified?

8) P24 — Why is reagent (b) (0.1m citrate buffer) prepared? It is not used in the subsequent assay.

9) P27 — Assay xylose by the DNS method, it is simpler and safer than the Nelson reagent and saves having to prepare two lots of reagent to measure the same thing (reducing sugar).

10) P25, 29 etc — The importance of thorough mixing of test solution with reagents prior to colour development MUST be stressed.

12) P38 — Are the air circulation and fresh air blowers fitted with filters? They must be, and ideally the exhaust air should be filtered but I do not think that this is too important here.

13) P43, Table 3. — Is the autoclave self-heating? If so by what? If not then is a steam generator needed? This point is dealt with later in the specification of the factory, but needs to be mentioned here.

14) Pectinase — (and/or pectin methylesterase). This (these) is/are not dealt with under the section on enzyme assays, yet we are agreed on the importance of pectin degrading enzymes for the success of the process.

15) The above comments are intended to be constructive, but the report as a whole is very sound. The design for the factory seems to be very good, with many similarities to our design for the soy sauce factory which we constructed in Cumbernauld, Scotland. Indeed, the whole report recalled many memories of

planning our factory.

Tne Ribbon Mixer is located in the Extraction Room but its most important job is mixing the bran with water, then mixing the sterile bran with inoculum. I would therefore suggest that it be located in the Processing Hall so as to limit the movement of sterile and inoculated Koji, thus minimising the danger of contamination. Furthermore only part of the finished koji will go for extraction with water, and on these grounds also it makes sense to re-locate the Ribbon Mixer, as this will result in the greater distance being moved by the smaller amount of material.

16) I am not persuaded that the proposed method of sterilizing and inoculating the koji is the best way to proceed. Filling trays with moist bran, sterilizing them, emptying them mixing with the spore inoculum, then refilling the trays with the inoculated material seems to be laborious and to involve a very high risk of contaminating the koji with undesirable organisms. Ideally the bran would be sterilized in bulk using a rotating autoclave then cooled in a current of filtered air, inoculated as at present, then spread into trays for incubation. However, the cost of rotating autoclave and forced aeration cooler would probably be too great for the project. I therefore, venture to suggest that, after sterilization and cooling, the trays are inoculated directly with a spray of spores suspended in water plus Tween detergent, then stirred with a hand-rake to disperse the spores through the bran. This point was discussed at CFTRI Mysore (see Appendix B).

17) It seems to be a little inconvenient to use the proposed arrangement for packaging and storing the dried moulded koji. Ideally, material should flow through the factory, with raw material entering at one end and finished product leaving at the other end.

Proposals for achieving these objectives were discussed with Dr Ghosh and Mr Atkinson and the agreed proposals were attached in a note to the blueprint of the factory layout which is held at IJIRA.

18) The staff toilet area might also have lockers where they can

deposit their ordinary clothing when they change into working clothes. I do not know how such things are handled in India, but we found it essential to provide the workers with some place to make drinks and eat sandwiches. Our Health and Safety at Work regulations also compelled the provision of a shower area for worker's use in the event of accidental contamination, and for those workers handling dry and dusty material to take a shower at the end of the day's work. Possible locations for such a shower have been identified on the work floor.

## APPENDIX B

## Report on visit to Central Food Technological Research Institute, Mysore

This visit, although arranged at very short notice was very productive. Discussions with CFTRI staff enable me to understand the details of the project much more clearly and so enable me to make a more accurate assessment of aspects which had puzzled me before the visit.

1. Design of Koji Trays

This was the most important matter which we discussed.

i) The metal sheet used must be the lightest available grade which will give trays possessing sufficient rigidity to withstand repeated handling when full of koji. It was agreed to make a tray from 1.6mm sheet for assessment.

ii) The trays which we were shown were too shallow. This apparently resulted from bending the sides double to give extra strength. The actual DEPTH of the FINISHED side must be sufficient to accommodate a layer of koji up to 3cm thick. For safety, and to permit mixing the contents of trays without spilling their contents, tray sides should be 3.5 to 4cm deep.

iii) CFTRI scientists reported that their experiments showed no difference between fungal growth rates in trays with bottom air holes and growth rates in solid trays. This surprised me until I examined their trays which were drilled with one small hole every 2cm or so. Even had the holes remained unblocked they would have made no significant contribution to the air supply to the koji; in fact, as the CFTRI scientists pointed out, the holes tended to become blocked with starchy material from the wheat bran. All published work agrees that good access of air to the bottom of the koji is essential for optimum mould growth and enzyme production (Fukushima, 1989). It is therefore essential that the koji trays have as many holes (and as large holes possible without loss of bran) as possible drilled into the still prefer trays made

with expanded metal or mesh, as this gives maximum aeration.

2. Mixing koji

If possible, the koji should be mixed at least once during the course of the fermentation (Fukushima; Ebine, 1989). We agreed that the easiest way to do this was to run a small rake to and fro across each tray. The teeth of the rake will need to be sterilised with alcohol before beginning each raking session, and thoroughly washed after each session.

3. Inoculation

It was agreed that the proposed method, involving sterilizing the bran in trays, emptying the trays into the ribbon mixer, mixing in the spore inoculum and refilling the trays was laborious and carried a high risk of infection. The equipment manufacturer suggested a method of inoculating the trays directly and this will be tested in an experimental rig which he will construct. If his idea is a success, and it seemed very sound to me, I think that it will find application in other solid substrate fermentations, with a possible export market. If built the inoculator will be installed in the process area, convenient for the koji incubators.

4. Location of Ribbon Mixer

It was agreed that the best location for it is in the production area, convenient for the autoclave.

5. Location of Steam Generator

This is now to be located in a lean-to outside the factory. This will make for a much better working environment and create needed space in the processing hall (see 3 and 4, above).

6. Equipment Supplier

I was very favourably impressed with Mrhopye Scientific Company, who are progressive, imaginative and strongly committed to our project.

7. Protective Equipment

All agreed on the danger of inhaling spores and koji dust. Filters (masks) are however uncomfortable to wear for long

periods and it will be difficult to get process workers to use them. Beards are thought to reduce the effectiveness of filter masks. Good positive ventilation of the packing area and koji inoculation area are therefore essential. Full head covers ("space helmets") with positive-pressure filtered air supply should be considered for workers at most risk of exposure.

8. Next Visit

It is desirable that I should have a rather longer visit to Mysore when I next come to Calcutta. Dr Ghosh should accompany me for part of the visit if he can be spared. Staff at CFTRI also wish me to give lectures to students on their post-graduate courses while I am there, as well as working in the solid substrate fermentation laboratory. The lectures are not germaine to the UNIDO IJIRA project, but I see them as a good public relations exercise.

9. Future Developments

I think that this project has a good potential to tap markets for enzymes other than the jute mills. I was very interested by the range of enzymes for which CFTRI have SSF processes (amylases, pectinases, proteinases), saw packaged enzyme solid and liquid preparations and alcohol make from starch, and sampled an excellent and crystal-clear banana juice (a notoriously difficult extract to clarify) prepared using their enzymes. There is great scope for transfer of their technology to the IJIRA production facility.

Company

Murhopye Scientific Company
B-11 Metagalli Industrial Estate
MYSORE-570016
Karnataka

Managing Director: Mr M G Byndoor.

References

EBINE, H. (1989). Industrialization of Japanese Miso Fermentation. IN Industrialization of Indigenous Fermented Foods, (Editor Steinkraus, K.H.) Marcel Dekker Inc, New York, p109.

FUKUSHIMA, D. (1989). Idustrialization of fermented Soy Sauce Production Centering Around Japanese Shoyu. Ibid, pp37 (effect of aeration), 35 (effect of stirring).

## APPENDIX C

### Areas for research

1. Production of improved strains of mould i.e. higher enzyme production and/or more balanced enzyme production. Pectinases and hemicellulases are particularly important.

2. What changes actually take place in TKP during enzyme treatment? What is the optimum balance of enzymes? Is it just proteolysis which matters or are other changes also important?

3. How desirable is a degree of cellulase activity (a) in jute preparation; (b) in TKP paste production by the improved process?

4. What is the cellulose inhibitor?

5. Why does enzyme treatment give brighter fibres; are there organic acids and/or other (chelating?) agents present and contributing to the effect or is it simply that the enzyme complex itself is cleaning the fibres more thoroughly? If factors other than enzymes are involved, can they be identified and (if necessary) be prepared by fermentation then added to the mixture or their production in the enzyme koji process be optimised?

6. Tannase. Since tannins are important colouring matters in jute and are responsible for darkening in response to sunlight, it would be desirable to remove tannins as far as possible consistent with process economics. Can tannases be produced using koji? If so, how can their production be integrated with enzyme production and how can they best be employed in jute processing?

7. Certain Basidiomycete fungi cause extensive breakdown of the jute fibre and this has been associated with the formation of oxalic acid by the fungus (although I am not clear as to the precise extent to which a casual connection has been demonstrated). Biodegradation of waste cellulosic materials to yield fermentable

carbohydrates is the subject of much research, but the great resistance to hydrolysis of the intact (crystalline) alpha-cellulose molecule plus the further protection given by sheaths of lignocellulose means that the production of fermentable carbohydrates from cellulose has never been economically viable tc date except in special circumstances. This is in sharp contrast to starch which is much less abundant in nature than is cellulose, and, because it has many industrial and technological uses, commands a substantial market price; whereas cellulosic materials, even those such as paper which have undergone considerable processing, are wastes which often incur a cost for their disposal, and have at most a small value as raw materials for recycling. Despite these factors, starch is extensively used as a substrate for fermentation to alcohol and other organic compounds, and also for production of high fructose syrups; the Japanese are interested in producing starch crops in Malaysia for fuel alcohol, and the production of ethylene for subsequent condensation into polyethylene is said to be a reasonable proposal by some authorities. Thus the potential for fermentable sugars from cellulose is enormous, and developing countries with their abundance of cellulose are particularly likely to benefit from any process which would yield hydrolysis under economically viable conditions.

This fungus grows under normal conditions of temperature etc., and oxalic acid, while a strong acid in comparison with other organic acids, is weaker than typical mineral acids;

| Acid | pH of 0.1 N solutions* |
|---|---|
| Oxalic | 1.6 |
| Acetic | 2.4 |
| Formic | 2.3 |
| Citric | 2.2 |
| Malic | 2.2 |
| Hydrochloric | 1.1 |
| Orthophosphoric | 1.5 |

*i.e. solutions giving equal numbers of dissociable hydrogen ions.

Thus it is unlikely that the breakdown of jute can be ascribed exclusively to the acidic nature of oxalic acid. The questions for research are (a) does oxalic acid aid hydrolysis of cellulose in some way other than simple lowering of pH; (b) does the fungus have any unusual cellulase (active at low pH for example)?

8. Waste from the jute mills is returned to the soil but is dusty to handle and would probably create a nitrogen deficiency when first added to the soil. IJIRA has an excellent biogas production programme using the waste, but I have the impression that this will only handle a part of it. Composting the waste would reduce bulk, correct the carbon/nitrogen imbalance and possibly produce a material which less disagreeable to handle. Such a product could well have a sale value as a soil improver and for use in seed and potting composts.

A more long-term but potentially profitable project is to study using jute wastes for growing edible mushrooms, as is already done very successfully with cotton wastes.

9. Problems relating to the koji process

i) The correct amount of nitrogen

For TKP improvement the production of proteinases by the mould is thought to be important, but for treating jute it is not needed. Enrichment of the wheat bran with an ammonium salt could therefore be beneficial in optimising carbohydrase production.

ii) Optimum water content in bran

The existing level is reported to be 39.2% made up as follows:-

Bran 100g contains 8.8g water

Water 50g

Total water in 150g koji base = 58.8g

The net effect of water uptake during sterilisation of the koji and loss during subsequent cooling are not recorded. Other workers report that wheat bran koji requires 41.5% W/W water for best mould growth. Consideration of water activity at these concentrations suggests that the difference although small may be significant, so the optimum water content should be determined.

iii) Effect of supplementing with other nutrients

Phosphate must be examined. Some workers add a small amount of magnesium sulphate, for magnesium, sulphate, or both is not clear.

iv) Acidification

In other koji processes the addition of a small amount of acetic acid has been shown to decrease the growth of contaminant bacteria and some results show an enhancement of fungal growth.

v) Oxygen

The need for greatly improved koji trays is an absolute essential, but even with forced aeration the literature indicates that the oxygen tension in normal air is too low for maximum fungal growth, and that enrichment up to 50% oxygen u/v is beneficial. I am very interested in the application of commercially available hollow-fibre devices for supplying the enriched air. As an additional benefit these devices necessarily generate a nitrogen stream which might aid in suppressing pests in stored wheat bran.