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JUTE RESEARCH AND DEVELOPMENT

DP/IND/86/037/11-02

INDIA

Technical report: Biosoftening of Jute*
(Second 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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United Nations Industrial Development Organization
Vienna

* This document has not been edited.

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ABSTRACT

Jute Research and Development: Biosoftening of Jute.
DP/INDF/86/0371/11-02/J13102.

This Report refers to the second visit to the Indian Jute Industries Research Association (IJIRA), Calcutta, by Dr Brian J.B. Wood. The visit to India occupied a total of five weeks including the initial briefing in New Delhi, and a visit to the Central Food Technological Research Institute (CFTRI), Mysore, which included lectures to their Faculty and students as well as very detailed discussions with the relevant personnel on aspects of the design of the koji plant, and also provided an opportunity to visit the Bangalore office of Millipore (India) Ltd for detailed discussion on the choice of ultra-filtration unit for concentrating enzyme extracts for use in jute biosoftening and biosolubilising Tamarind Kernel Powder (TKP). A third visit will be necessary to aid in commissioning the koji factory. Even closer cooperation between CFTRI and IJIRA is recommended. All present and proposed applications of enzyme extracts in the jute industry are discussed and evaluated, and the need for parallel studies of microbiological changes during applications of enzyme production are discussed. Areas for research are listed and priorities are suggested. Two jute mills were visited, their production and utilisation of enzymes inspected, and improvements discussed with their managerial staff. There can be no doubt about the great enthusiasm for using enzymes among staff in mills which have adopted this technology.

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I. INTRODUCTION

This report describes my second visit to IJIRA under the Biosoftening of Jute element of the UNIDO programme to IJIRA. It comprises three parts; first a substantive report, second a listing of activities and third are my summarised recommendations for future development of the project.

II. SUBSTANTIVE REPORT

A. Timing of Visit

At the end of my first visit, I recommended that I make only one further visit and that this be timed so that I could take an active part in starting the production of enzyme-mixture at the koji factory being built at Kinnison Mill. From my first visit, I was well aware that things were moving very slowly there and when the question of the date for my second visit was being discussed, I made a tentative suggestion of September/October 1990. However, IJIRA pressed August/early September upon me, which I accepted with considerable misgivings. I arrived in Calcutta to find that the koji plant was a long way short of being ready to have the electrical supplies, equipment, etc., installed, let alone trying experimental production runs. My misgivings about an August visit, although principally predicated upon my doubts about the possibility of the Kinnison koji plant being ready to operate that early, also reflected my concern that the Monsoon period would present difficulties in moving around, plus an increased risk of disease. The two latter fears proved as well founded as the first, with flooding (and strikes) creating problems, and with an enteric amoebal infection making a contribution

to my stay as memorable as it was unwelcome. I have still received no adequate explanation for this timing of my visit.

As indicated above, it was my clear intention that my second visit to IJIRA be my final one on this project, and I had stated this in my letters to UNIDO, Vienna.

I have many other commitments, and as a University lecturer I depend heavily upon the generous goodwill and cooperation of my colleagues to fit activities such as the IJIRA project into my schedule; such cooperation and goodwill although extensive are not infinite. It is therefore with considerable misgivings, and only after the most thorough discussions with the Chief Technical Advisor, Mr R Atkinson, that I have agreed to make a third visit, WHEN THE KOJI FACTORY IS READY TO BEGIN. Agreement to this is purely because of my commitment to the project. This visit will encompass a maximum of one working week in Calcutta and I hope that when exact dates come to be discussed I shall on this occasion be advised of such matters as local holidays; during the first full week in Calcutta of the present visit there were two public holidays, and with a one-day strike thrown in this meant that I had only 2 days at IJIRA.

A tentative date in late February 1991 has been suggested for this third visit. If the stipulations made above can be adhered to, then this date will fit conveniently with an invitation to be the Foreign Guest Instructor at an All-India Workshop on Solid Substrate Fermentation in CFTRI Mysore, where I would of course, plan to feature this IJIRA programme as an imaginative and innovative use of SSF and enzymes in my contributions at the Workshop.

In order to reduce the time-wasting and stressful travel between the

centre of Calcutta and Kinnison Mill, and recognising that I will need to spend several days at the koji factory, I am willing (not without some misgivings) to stay on site for a few days, provided that appropriate safe, secure and comfortable accommodation, with suitable water (sterile) and food, can be provided, together with ready access to help in the event of medical or other emergency.

B. Choice of Organism(s) for Enzyme Production

The work to date has been exclusively with a strain of the filamentous mould Aspergillus terreus originally isolated at IJIRA. In practice this organism has been quite successful at producing an enzyme mixture which serves for both biosoftening and upgrading the raw jute and the solubilisation of tamarind kernel powder (TKP) which is used as a size when weaving jute into textiles. However, this should not be taken to suggest that the IJIRA strain of A. terreus is necessarily the best or the only mould for these purposes.

1. Safety

I accept without any reservation IJIRA'S assurances that under the operating conditions and with the batch sizes of koji which they have employed, both at their laboratories and in the mills which are producing koji for their own use, no health problems have been observed. On the other hand, staff at CFTRI report that several of their workers suffered respiratory and other allergic effects consequent upon working with production batches of the koji. In a few cases the symptoms were apparently sufficiently serious to require hospital treatment. CFTRI had recorded their concern over this in writing in Part 9 (Recommendations) of their Report to IJIRA on their

investigations of the enzyme koji process. I examined a copy of that Report during my first visit to Calcutta, and my First Visit Report to UNIDO discusses several matters arising from the CFTRI Report. Consequently, I am rather surprised, given my interests in safety matters, that I overlooked this important problem, which is very clearly delineated by CFTRI, and I agree that I may be guilty of carelessness in my reading of that report, but I also feel that IJIRA staff ought to have drawn my attention to the CFTRI staff's views on the matter, even if only to emphasise their rejection of those views. I do not have sufficient data for proposing a reconciliation of, or an adjudication between, these two opinions on the safety of the A. terreus koji. I am however certain that a solution satisfactory to all parties must be found. At the very least this will have to entail modifications improving the collection and filtration of the air exhausted from the koji production rooms and the rooms for drying and packaging dry koji and extracting moist koji in the preparation of liquid enzyme concentrate. Proposals to give effect to this were agreed during the visit to CFTRI by Dr Sinha and myself.

2. Enzyme Yield

CFTRI staff indicate that they have moulds which will give substantially higher yields per kg. koji of the various enzymes of interest which are produced by A. terreus than the latter does. They are also more confident about the safety of these alternate organisms, and could supply moulds with high production of pectin-hydrolysing enzymes, which I regard as very important, but which are apparently absent from the A. terreus cultures. ON THE OTHER HAND the IJIRA A. terreus strain is the only organism which is known to produce a

substance possessing the vital property of specifically inhibiting "cotton cellulase" activity (that is to say the degradation of intact cellulase fibres, leading to weakening of the jute thread) while permitting B-glucanase activities essential for upgrading the jute to continue acting without check. Their evidence shows that the inhibitor works on "cotton cellulase" from sources other than A. terreus, and they seem to be fairly close to identifying it as a rather simple chemical compound. I advise that they complete identifying the inhibitor as rapidly as possible, then if it is a readily available substance (or mixture) that they use a better mould source of cellulase and add the inhibitor to the extracted cellulase before applying it to the jute.

CFTRI have indicated to me that they can supply mould strains which will make all the enzymes needed for the IJIRA process in good yields, and which would simply require combining the various liquid enzyme extracts in appropriate proportions to yield an optimum balance of enzymes for a particular task, be it biosoftening retted jute, biosolubilising TKP or enzyme retting of fresh jute which has been mechanically stripped from the jute "stick". This last process, although it will require considerable development is attractive in that it could -

- (a) eliminate the severe water pollution caused by conventional retting;
- (b) possibly yield a liquid by-product useful as a feed for farm animals.

C. Chemical Softening of Jute

I learn that in parallel with the biosoftening programme, another

group at IJIRA report useful results from a Chemical Softening approach, in which soda ash, urea and a wetting agent are added to the raw jute during piling. Mr Atkinson and I have discussed this process and we suspect that it may work by providing extra nitrogen (urea), minerals and mild buffering against acids produced during piling (soda ash), so enhancing the activities of the bacteria which are known to grow during piling, and which are reported to be promoted by the release of fermentable carbohydrates during enzyme biosoftening. Retting will ensure that the resulting jute fibres will be very short of assimilable nitrogen, phosphorus and probably some trace elements. The effects of the chemical softening process on the development of bacteria during piling must be examined urgently. A combination of chemical and biosoftening might give very interesting results.

D. Tamarid Kernel Powder

This was a new material to me and I have therefore obtained a computer-search printout of all references to it in "Chemical Abstracts" for the past ten years. A number of these refer to textile uses of TKP, ways of preparing cold-water-dispersable TKP, and evidence that TKP has some undesirable effects in animals' diets (thus reducing or even negating its value as a food constituent); this is favourable for our purposes by decreasing the likely price. This file has been left with IJIRA staff.

E. Bran

I had been puzzled by the rather low levels of water used in the IJIRA kojis, and directed attention to the fact that it was sub-optimal according to published data (see my previous report). I now learn that the bran most readily available to IJIRA is of poor quality,

being the product of worn-out mill equipment which can no longer remove endosperm efficiently, resulting in excessive amounts of starch and protein in the bran. Not only does this decrease the amount of water which the bran will absorb before clumping, but the presence of starch and protein will divert the mould's energies away from making the enzymes required for jute treatment. I strongly support CFTRI's view that koji process must use a high grade of bran. Every effort must be made to find a good flour mill within a reasonable distance from Calcutta. Shipping bran from Mysore would be prohibitively expensive, but it might be interesting to estimate the cost to IJIRA of a small modern, high-efficiency flour mill and the profit from the sale of premium quality flour which it would produce. Dr Chakrabarti's work has shown that manganous ion is a powerful activator for the proteinase produced by A. terreus, and an experiment in which one set of koji trays were supplemented with manganese while a control set was treated normally suggested that the supplementation stimulated mould growth; this result must be confirmed and the effect on enzyme levels determined. If benefit to enzyme production is found then routine supplementation must be considered. CFTRI report that supplementation of their bran with a mixture of minerals increases enzyme yields in some systems up to three-fold. Their supplementation mixture must be tried with A. terreus, if this is to be the mould used in practice. Where enzymes are being produced for jute softening, the addition of ammonia to the koji may be beneficial by reducing the need for the mould to produce proteinases. Ultimately I believe that the production of enzyme mixture for TKP paste preparation and for jute softening must be treated as separate

tasks requiring different growing conditions and possibly different moulds or combinations of moulds.

F. Peroxide Bleaching of Jute

It is reported that this is also improved by pre-treating the jute with enzymes. Pectinase is said to be important, but otherwise the enzymes are the same as these said to be required for jute softening, and I would try for a single blend of enzymes which met the requirements for both processes, particularly as I believe that pectinase will aid bio-softening. Enzyme treatment must precede bleaching, dying, etc., as it will increase the effectiveness of the latter processes by removing unwanted material from the jute fibres.

G. Optical Brightening of Fibres Tannase

Tannase (3.1.1.20) has been implicated in other studies as an enzyme with optical brightening activity, by reducing the amount of tannin bound to the jute fibre which would subsequently darken under the influence of light and air.

Commercial tannase, for use in beverage industries (notably instant tea where it aids solubilisation of the extract) is prepared from Aspergillus oryzae and A. niger, and it is therefore reasonable to postulate that it may also be present in our A. terreus. The organism is quite closely related to these others, and it is worth recording that A. niger and A. oryzae belong to quite distinct sub-groups of the genus, yet strains of both possess the activity in commercially useful amounts.

Tannin Acylhydrolase, E.C. Number 3.1.1.20, is most active in acid media (pH 3 to 5) and at temperatures 45 to 55°C. The fact that the commercial enzyme also contains Amylase, Glucoamylase, Protease and

Cellulase further emphasises the closeness to our preparation.

H. "In House" Production of Koji at Mills

Some mills prepare their own koji and the enzyme extract for use in bio-TKP production and biosoftening. From what I can gather as to the amounts being produced at the mills, I form the impression that this will continue to make a significant contribution to the total supply of enzyme extract available for use in the industry, even after the central koji factory is in full production. I feel that there is need to collect data on the total koji production "in house" at mills. In order to ensure that the mills producing their own koji and extract operate at optimum efficiency, I suggest that the following points be given consideration:-

1. Contamination

This seems to be a regular problem, with Rhizopus spp. (white, very fluffy growth topped with black spores) the offending organism. As is to be expected the problem is worse in the wet season than at drier times of the year. This problem will never be totally eliminated, but can be reduced by a few simple measures:-

- (i) The inoculation area should be screened from its surroundings as far as possible.
- (ii) Draughts in it should be kept to a minimum while inoculations are being done; for example unglazed openings to the outside should be fitted with temporary screens or baffles.
- (iii) The inoculation area must be reserved entirely for that purpose and must never be used for general storage; storing dusty, spore-rich materials such as hessian sacks of bran is particularly reprehensible.

- (iv) Particular attention to the area's cleanliness is vital, with walls as well as floors being frequently and thoroughly cleaned.
- (v) Prior to starting a set of inoculations the working surface must be cleaned and then swabbed down; I prefer a 70 : 30 ethanol water mixture for the latter job (care - no flames until the surface has dried and the alcohol fumes dispersed), but swabbing with diluted Dettol or other disinfectant is acceptable.
- (vi) Similarly, if the floor has a dusty surface, a light spraying with diluted disinfectant (or even plain water if disinfectant is unavailable) just before starting inoculation will be beneficial.
- (vii) I tend to repeat the swabbing of work surfaces at the end of a session of inoculation, and recommend this as a precautionary practice.

2. Design of Koji Trays

Once the mould spores have germinated, growth is rapid and considerable heat is produced. For good growth and also to help reduce overheating, an abundant supply of air is essential. The desired mould is strongly aerobic (oxygen-requiring) whereas the contaminant Rhizopus can grow well at low oxygen levels and so is aided in competing with and overgrowing A. terreus if oxygen becomes limiting. The trays being used at the factories which I have visited all have tight, close-fitting lids which severely restrict gas exchange. These lids must be replaced. I propose a design which is 1cm wider than the tray, fitted with three internal lugs to raise the

top of the lid 0.5cm or so off the lip of the tray and with sides 2cm deep to deflect moving air and keep contaminant spores out.

While good gas exchange in the area where the trays are incubated is obviously essential, draughts must be prevented from blowing directly onto the trays.

I. "Green" Jute and Jute "Stick"

1. Green Jute: I stripped the outer layers from a freshly cut jute stalk. Gentle scraping with the back of a knife removed much of the juice and soft tissue, leaving clean-looking, pale yellow-green fibres, still held in a matrix, but fairly easy to separate into fibres. This appeared to remove a fair amount of material which would otherwise need to be retted. The juice and soft tissue could be acid-fermented and used as an animal feed. The fibre looked to be in prime condition for enzyme "retting", and had not changed colour after 22 days in the light at ambient temperature.

Experiments to try:

- (i) Repeat, measuring weight-loss;
- (ii) Determine moisture content of the fibre portion;
Determine moisture content of a comparable piece of fresh bark;
- (iii) Try the effect of bleach on fresh fibre;
- (iv) Measure polyphenol oxidase as there seems to be very little browning of cut or bruised tissue, even at 30°C.

2. Stick: Dry, retted stick is rather like Balsa wood, soft, springy, spongy, very light in weight and with little mechanical strength. Fresh stick is somewhat similar but will presumably contain more by way of relatively easily fermentable material. Both were

easily cut up into 2-3mm cubes which looked like an excellent support for SSF.

Experiments to try:

- (i) Determine nitrogen content of each;
- (ii) Determine lignin content of each;
- (iii) Determine reducing sugar content of each;
- (iv) Determine water uptake of dry material;
- (v) Cube each, add nitrogen to same final level in each, plus mixed nutrient salts and some glucose for the retted stick, sterilise, inoculate with A. terreus and measure growth and enzyme production.

J. Concentration of Enzyme Extract

As my understanding of the project has grown, I have moved from my initial skepticism about the merits of the distribution of enzyme extract in liquid form and now feel that, with proper storage, it will be better than sending dry koji to the mills for various reasons. Paramount is safety; the reports of allergic reactions by CFTRI staff to koji worry me and I feel that by extracting the still moist, freshly harvested koji, the exposure of workers to dust and spores within the koji factory will be much reduced, while workers in the customer mills will not be exposed at all.

The mill using the enzymes will find diluting and spraying a concentrate much more convenient than extracting dried koji, filtering, standardising the extract, then using it; also they will not need to provide the protective equipment needed for workers exposed to dry koji. Remarkably, benefits when upgrading jute are reported from sprinkling the koji residue (after enzyme has been

extracted) onto raw jute. It is not clear to me as to how much of the extracted koji could be used by Kinnison Mill, but this question is not without importance, both because it establishes a commercial value for the extracted koji, and because this application helps in the disposal of the solid residues after enzymes have been extracted. These questions need to be addressed by IJIRA.

Selection of equipment to concentrate the enzyme extract to 10% of its original volume by ultra-filtration was already being discussed with equipment suppliers when I arrived in Calcutta, and Millipore were emerging as offering the most suitable equipment for the project. I was involved in three discussions with IJIRA and Millipore staff, first at IJIRA (10th August), then informally over dinner in Bangalore (25th August) and finally at a very interesting meeting in Millipore's Bangalore office (Sunday, 26th August). The equipment recommended by Millipore, while seeming rather expensive, should do the job of concentration with high efficiency and at the usual Millipore standards of reliability. Funds for the purchase should be made available by re-routing funds not required elsewhere.

The enzyme concentrate is of course very unstable and liable both to attack by microorganisms and to auto-digestion of the active enzymes by the proteinases present in the extract. I am told that the latter is not a serious problem but I suspend judgement until I see experimental results pertaining to it. The basic need is for storage under refrigeration to slow both forms of biodegradation, backed up with an effective biocide to prevent microbial growth; toluene is cited in the proposals but I regard this as a hazard for the workers handling the enzyme extract, and it can be of limited efficiency, with

records of bacteria growing in the water beneath a film of toluene. Toluene may also weaken some plastics used for concentrate containers. I therefore advise that a modern, safe, water-soluble, broad-spectrum biocide be obtained from one of the specialist suppliers and used in place of toluene.

K. Role of Oxygen in the "Piling" Stage of Jute Preparation

It is clear beyond any reasonable doubt that there is considerable growth of microbes during the period when the raw jute is held in piles; the simplest and most obvious proof of this is the way in which the piles heat up quite soon after they have been made and covered, recalling the behaviour of a garden compost heap. I have heard mention of piling methods in which the jute is piled onto a slatted surface, thus giving some access of air from the bottom of the pile, again reminiscent of some composting techniques, but I have not found any evidence that there has been any proper scientific study of such aerobic piling. Such a study would be very easy to do and should include microbiological monitoring of the aerobic piles and control conventional piles, as well as assessments of quality of the jute resulting from the two processes. It could be that forced aeration of the pile may be beneficial in accelerating the process and controlling the pile's temperature and humidity. I am coming to appreciate piling as a solid substrate fermentation, and therefore believe that it must benefit from being treated as such and being given proper environmental control.

L. Comments on Items in my First Mission Report

Page 8, Item III 3. I have had no reply from Poland about my request for high-pectinase mould strains, (despite a follow-up letter) but I

now realise that CFTRI are willing and able to supply excellent cultures possessing any attribute required for this programme.

Appendix A. Item 1. No answer received to this.

Item 3. Agreed to by IJIRA and CFTRI.

Item 4. Dealt with in the Substantive report, Section IIE

Item 5. Agreed by all concerned.

Item 6. Resolved to my satisfaction.

Item 7. Apparently at the Centrifuge, but this must be written into the protocol.

Item 8. To be deleted from the protocol.

Item 9, 10. Protocols to be corrected to take account of these points..

Item 12. I now regard the filtration of exhaust air as being VERY important (see Section IIB 1). Answers to the other points have not been received.

Item 13, 14. I stress these points again.

Item 15. Re-location of the Ribbon Mixer agreed to.

Item 16. Agreed to, and appropriate modifications have been made to the design of equipment and the protocols for its use.

Item 17. A reallocation of room functions has been made.

Item 18. Changes have been made where these accord with Indian practice.

Appendix B. Some changes have been made to the design of trays, I have been assured, but they were in a locked store at Kinnison Mill, so I was unable to inspect and evaluate the modified trays. Mr

Byndoor is also constructing a tray (or trays?) with a mesh base, to assess the validity of my views on the superiority of this design.

Appendix C, Item 1. I am now certain that improved enzyme output can be obtained by the simple expedient of accepting advice and strains from CFTRI. Their organisms will be superior because they are selected for optimum growth by SSF, whereas imported strains will most likely be for use in liquid fermentations. There is absolutely NO place within this programme for experiments in breeding new strains of mould and even less case for "genetic engineering", a thoroughly unproven technology for this type of PRACTICAL programme.

Items 2, 3, 4, 5, 6 are still important. A few answers have turned up. For example it seems quite possible that tannase may be produced by A. terreus, but a definitive answer is needed. As will be clear from the rest of this report, I still think that these are crucial points for the success of the programme.

Item 8. It seems that most of the jute waste is in practice either burned to help raise steam or sold to particle board manufacturers. I have learned that the mineral oil content makes jute waste unsuitable for use in growing mushrooms; a pity. I suspect that the same problem would cause difficulties in using it in composting.

Item 9. The various points under this heading continue to be important. Nos. (i), (ii) and (iii) are mentioned elsewhere in this report and demand further investigation, as does (iv). I now think that oxygen supplementation would create problems with heat removal from the koji, and so is not useful in practice.

M. British Council Links

The opportunity arose to visit the British Council Offices in Calcutta

with Dr Sinha. This was planned as a discussion of my then forthcoming visit to CFTRI, as I had formed the impression that IJIRA would not qualify as a partner under the rather strict rules governing exchange schemes. However, to the surprise and delight of Dr Sinha and myself the British Council staff showed interest in the relationship which my participation in the UNIDO programme had inevitably caused to develop between the University of Strathclyde (my employer) and IJIRA. While they were not able to give us a definitive ruling on the matter, they felt that the role of IJIRA within CSIR, its attachment to the Indian Ministry of Commerce and its links with Calcutta University could justify treating it as a body with an educational component to its activities. As such it could qualify for participation in some exchange arrangements covering the secondment of IJIRA staff to the UK for training, and also visits by Strathclyde University academics to IJIRA. Dr Sinha and I are preparing a paper for consideration by the British Council and we see this as a way to develop the longer term research which we regard as necessary for a more complete understanding of the scientific aspects of the present UNIDO programme, but which cannot be justified within the essentially practical, mission-orientated organisation of that programme. We shall of course keep UNIDO informed of developments in this matter.

Our contacts at the Calcutta offices of the British Council were:

Dr Philip French, First Secretary (Education and Science);

Ms Veena Lakhumalari, Projects Officer;

Dr Ambar Ghose, Senior Projects Officer;

British Deputy High Commission, British Council Division,
 5 Shakespeare Sarani, CALCUTTA, 7000,071
 Telex, 215984 or 213131 BCCA IN
 FAX, 91 33 444804 or 224804.

III.

ACTIVITIES REPORT

Date	Activity
August	
Sunday 5th	Depart U K
Monday 6th	Arrive Delhi. Bag missing; passport endorsed - visa allegedly expired. Visit UNDP H.Q.
Tuesday 7th	To UNDP H.Q. re payment, then to Immigration Office. Visa confirmed O.K. Return to UNDP. Finally collect money after much delay. Lunch at UNESCO. Go to hotel to deposit money, then to airport to collect baggage (UNDP had received confirmation of its arrival).
Wednesday 8th	Go to Agra, see Taj Mahal and Red Fort.
Thursday 9th	On to Calcutta and to IJIRA. Met by Dr Sinha. Meet staff, settle into office, receive progress report.
Friday 10th	Discuss project and my programme with Dr Singha. Taken ill at midday and return to hotel.
Saturday 11th	At hotel suffering from acute digestive tract
Sunday 12th	upset. Recovered enough by Monday to complete
Monday 13th	review of the IJIRA progress report.
(Hindu holiday)	
Tuesday 14th	At work. Discussed membrane filter methods for

concentrating koji extract with Dr Sinha and Millipore Company Rep in the morning. Saw Company Medic re intestinal problems. Discussed his report with Dr Singha in the afternoon.

- Wednesday 15th National day holiday. Wrote note on Tannase.
- Thursday 16th Strike.
- Friday 17th At work. Examined samples of stick both retted and from fresh jute. Examined bark layer from fresh jute.
- Monday 20th Visited British Council to discuss project prospects re CFTRI and IJIRA. Meetings with Drs S.K. Chakrabarti (a.m.) and Sinha (p.m.) to discuss aspects of their contributions to the programme.
- Tuesday 21st Visited Kinnison Mill to inspect the koji factory, and I was also shown round the mill and their own small enzyme production unit. The latter's output of enzyme for TKP modification was directed into TKP for high quality products and was insufficient to meet the requirements of TKP for sizing carpet-backing and similar grades of products.
- Wednesday 22nd Reported to Mr Atkinson on my work to date; tried to make appointment with Doctor, reviewed progress on effect of manganese on koji with Dr Charkrabarti. Completed arrangements for visit to Mysore. Visited British Council with Dr Sinha to discuss possible aid on projects.
- Thursday 23rd Shopping, paperwork; discussions about Dr Sinha's

slides on application of enzymes in jute mills; 4pm meeting with Drs Sinha, Ghose and Mr Atkinson to review progress on biosoftening, identify problems and priorities for investigation, consider the current state of the koji factory.

- Friday 24th Completed revision of their MS on effect of cellulase inhibitor on the effect of enzyme extract on jute fibrils. Discussed relationship of chemical and biological softening of jute with Mr Atkinson. We agreed that there is scope for reconciling the two methods and that a meeting on this topic is essential. Dealt with travel matters through Mr Chakravarty. Began work on my reports. Drafted outline of a proposal for application to the British Council to support collaboration on basic scientific data relevant to this programme.
- Saturday 25th Travelled to Bangalore. Dinner with Millipore.
- Sunday 26th A.M. meeting with Millipore; P.M. travelled to Mysore.
- Monday 27th Met with Professor Karanth and staff - my letters not received. seminars hastily planned. long meeting re project - see notes. Ended 7.30pm. Many problems with system and information.
- Tuesday 28th A.M. Discussions re koji plant and visit to microbiology. P.M. Lecture 1 - soy sauce.
- Wednesday 29th A.M. Talked with Karanth (Salford PhD) and SSF group. Session with PG students. Further talks

regarding problems of the IJIRA programme. Visited Food Science.

P.M. Lecture on Applications of SSF in the production of food. Invited to Conference February 1991.

- Thursday 30th A.M. Manpower Development, Oilseed Technology, Algal Biotechnology
P.M. Lecture on preparing scientific MSS.
- Friday 31st Return to Calcutta.
- Saturday 1st Shopping. 6p.m. lactic acid meeting.
- Sunday 2nd
- Monday 3rd Visit Birla Mill - Depart 8.30am. - cancelled. No work (1 day strike). Worked at hotel on writing my Technical Report.
- Tuesday 4th Visited India Mill. Interesting experiments on carpet production. Made suggestions concerning some improvements in their rather good facility for producing enzymes on site.
- Wednesday 5th Reviewed data on chemical softening/upgrading of jute. worked on Technical Report.
- Thursday 6th Worked on Technical Report in the morning, and early afternoon, then was to visit Calcutta University with Dr Sinha. We had planned that I give a lecture on the Biotechnology of Soy Milk and other Soy Food products, but the many interruptions to the students' studies caused by the recent strikes and civil disturbances meant that they had

Friday 7th

too much basic work to do for them to be able to attend an outside lecture arranged at short notice.

A.M. Visited UNDP office in Calcutta. Final visit to Bank. Saw Sinha and graduates who are interested in coming to U.K. and discussed their backgrounds, interests and the kind of work which they could do at Strathclyde. One is interested in assaying enzymes and could usefully extend the range of assays to include pectin hydrolysis and tannase. The other man works on TKP and might extend the knowledge of proteins in the TKP and the effects of different types of proteolysis on their capacity to glue jute fibres together. Both have responsibilities for IJIRA's culture collection and would benefit from some time spent learning how we store and handle cultures, especially the use of techniques for long term storage such as lyophilisation and storing mould spores in sterile soil.

P.M. Informal meeting of Mr Atkinson, self and senior staff from the jute biosoftening and chemical softening groups to establish what common ground exists here.

IV. RECOMMENDATIONS ON PRIORITY PROBLEMS

Many recommendations are made through the substantive report and I do not intend to list them all here, although each is important in its context. This listing (in an approximately descending order or

priority) highlights some of the more immediate problems.

1. STARTING PRODUCTION AT THE KOJI FACTORY has absolute priority over every other matter.
2. Exact chemical identification of the cotton cellulase inhibitor discovered by Ghose et al and determination of its molar activity against the enzyme, as a guide to the working concentrations needed in prepared, balanced enzyme mixture. Is it undesirable in preparations for bio-solubilising TKP?
3. Effect of supplementing bran with trace minerals on growth of and enzyme production by mould. Use of high quality bran.
4. Producing improved design koji trays for use in mills' "in house" enzyme production, and evaluating the performance of these trays.
5. Growing pectinase/pectinmethylesterase producing moulds (from CFTRI) and determining the value of these enzymes in the biosoftening and upgrading of jute.
6. Estimating tannase (if any) in the present enzyme extract and examining the effect of adding extra tannase (prepared by SSF of mould strains supplied by CFTRI) on the brightening and lightening of biosoftened jute.
7. Comparative microbiological and quality studies of piled jute from (a) conventional piles; (b) piles treated with enzyme; (c) piles treated with extracted koji; (d) piles treated by the "chemical softening" process; (e) piles treated with a combination of (b) or (c) with (d); (e) "aerobic" piles.
8. Blending enzyme extracts obtained from CFTRI mould strains selected for their high yield of specific enzyme(s). The IJIRA A. terreus strain has served this programme magnificently, and it, or a

more productive relative, will continue as the organism of choice for "in house" production of enzyme extract by Mills, subject to satisfactory health safeguards, but blending extracts from high-yielding moulds for specific purposes (TKP biosolubilisation, jute biosoftening, jute brightening and preparation for bleaching and dyeing) must be the favoured route for the controlled enzyme production facility, once confidence and experience in operating it for conventional enzyme production has been gained by all necessary staff.

9. A much more detailed understanding of the relative importance of the various changes which occur during biosolubilisation of TKP, leading to optimised enzyme mixtures for this purpose. Identifying the protein(s) important in sticking jute fibres together will permit optimising their degree of digestion and their concentration in the working size.

10. Enzyme "retting" of freshly harvested jute, peeled mechanically from the jute stick. This has got to come, as the pressure on water supplies and demand for higher water quality standards increase and force the rejection of conventional retting with its wastefulness of water and of jute biomass, and its enormous pollution of ponds and streams.