## OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

## DISCLAIMER



## FAIR USE POLICY



## CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

RESTRICTED

18902

DP/ID/SER.A/1460
10 April 1991
ORIGINAL: ENGLISH

JUTE RESEARCH AND DEVELOPMENT
DEVELOPMENT AND PROMOTION OF DIVERSIFIED END-USES OF JUTE
IJIRA, CALCUTTA
DP/IND/86/037/11-02/B/J13102

# Technical report - Biosoftening of jute*

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 S.K. Niyogi
Expert in purification, characterization, and
genetic engineering of enzymes

Backstopping officer: J.P. Moll, Agro-based Industries Branch

United Nations Industrial Development Organization
Vienna

*This document has not been edited.

V.91-23283

CONTENTS

# ABSTRACT

Jute Research and Development: Biomodification of Jute. DP/IND/86/11-02/B/J13102

This Report covers the first official visit to the Indian Jute Industries' Research Association, Calcutta, India, by Dr. Salil K. Niyogi. The visit lasted a little over two weeks, including two trips to three jute mills outside Calcutta and a one-day visit to the library of the Indian Institute of Chemical Biology, Calcutta. The report describes the applications of enzymes (derived from a moldy wheat bran extract) in upgrading the jute fibre and in enhancing the quality of tamarind kernel powder used for sizing of jute. The various methodological developments in these processes are discussed in detail along with suggestions for future research. The report also describes the visits to the jute mills where enzyme applications are being made. Interactions with the IJIRA research staff, particularly in regard to future research development, are described in detail. My contributions to the Project are described along with specific recommendations for future directions.

# I. GENERAL BACKGROUND

The jute industry has a long history and a rich past in India, particularly in the state of Bengal before independence. Partition led to the division of Bengal into West Bengal, an important agricultural and industrial state of India, and East Pakistan, which subsequently emerged as a separate state -- Bangladesh. Most of the good quality jute, which does not need much upgrading, is located in Bangladesh, whereas the jute mills located mostly in West Bengal were starved of good quality jute. Thus, upgrading and refining of the West Bengal jute are prerequisites for its further treatment.

Although the jute industry has fallen upon hard times during recent years, partly due to competition from plastic and other synthetic materials, several factors have become important in possibly ensuring a brighter future. Firstly, there are the obvious human and economic aspects. About 400,000 families in rural West Bengal depend upon jute farming and many thousands in the jute mills along the banks of the Hooghly river owe their livelihood to jute processing. Secondly, the establishment of and efforts by the Indian Jute Industries' Research Association (IJIRA) have been noteworthy and encouraging of a brighter future for the jute industry. Thirdly, the establishment of a United Nations Development Programme (UNDP) project at IJIRA led to the infusion of much needed funds and talents into IJIRA. Finally, it should be pointed out that both the Government of India and the industrial sector, acting in concert, have come forth with the necessary support and cooperation in reviving the jute industry.

# II. INTRODUCTION

## A. General Outline of Jute Processing

Jute plants are harvested close to the ground. They then undergo the process of "retting" by immersion in water. Retting separates the outer fibres and bark from the woody core. After washing and drying, the jute fibres are sent to the mills where they are graded and mixed for the proper blend. This is followed by stretching and impregnation with water and oil, all done mechanically, for the purpose of softening. The softened jute is piled and allowed to ferment by the consecutive action of naturally occurring aerobic and anaerobic bacteria. The jute is then processed through a series of machines to produce thread. The thread is sized, woven, finished, and finally baled for shipment.

## B. Use of Enzymes in Jute Industry

For a detailed description of the use of solid substrate fermentation of wheat bran by the mold, *A. terreus*, to produce enzymes for the jute industry, please see the report to UNIDO by Dr. B. J. B. Wood (December, 1989). Also described in that report are the plans for the construction of a small factory to produce the enzymes on a commercial scale. The latter plans were developed for IJIRA by the Central Food Technological Research Institute (CFTRI), Mysore, in a report entitled "Detailed Project Report on Establishment of Solid State Fermentation Culture Plant for Production of Enzyme Complex for Use in Jute Industry" (July, 1988). The extracted enzymes are used in two ways -- directly for upgrading or softening of jute and for biomodification of the sizing agent derived from tamarind kernel powder.

## C. Nature of Biosoftening and Biomodification Projects

### 1. Biosoftening of Jute

Direct application of the enzyme extract from moldy wheat bran leads to considerable upgrading of low quality coarse jute. The enzyme extract can also be used subsequently for increased bleaching and brightness of jute fabric. The mechanism of the latter processes is currently being investigated at IJIRA.

### 2. Biomodification of Sizing Agent

This involves biomodification of tamarind kernel powder (TKP) by an enzyme extract from moldy bran. Application of enzyme-treated TKP to jute fibres leads to improved sizing. TKP itself had effectively replaced starch as a sizing agent during World War II. Treatment of TKP with the enzyme extract facilitates the suspension of TKP in water, thereby eliminating the need for boiling it. This leads to considerable savings in manpower and steam. Enzyme-treated TKP has also been shown to be superior to unmodified TKP as a sizing agent.

In summary: (1) Profitable use of enzymes for upgrading of jute fibre; (2) Biosizing by use of Bio-TKP -- improved warp size and weaving, and coal savings of 25-30%.

Twelve mills have adopted biomodification. Central enzyme plant is at Kinnison Jute Mill.

## D. Roles of Cellulases and Proteases in Bioprocessing

Cellulases and related enzymes in the moldy bran extract are important in the biosoftening process; no role of proteases in this step. For biomodification of TKP, proteases are important; the presence of cellulase at this step is undesirable because it reduces the viscosity of TKP. Therefore methods for the separation of the above activities are important. These are discussed in detail below as areas for research development.

# III. AREAS FOR RESERACH DEVELOPMENT

## A. Development of Methods for Separation of Cellulase and Protease Activities

### 1. Physical Separation by Ultrafiltration

This is a popular procedure with wide-spread applications in industry. Various types of membrane filters with widely different porosities are available for separating proteins of different sizes. Some progress has been achieved at IIIRA through UNIDO fellowship training (see later).

### 2. Selective Precipitation of Specific Enzyme Activities

The various agents that can function in this process include polyethylene glycol 6000 (PEG 6000), polyacrylic acid, tannic acid, and cetyltrimethylammonium bromide (CTAB). Here again the selection of the reagent will depend upon its efficacy as well as its cost. For example, some of these compounds are byproducts of other industries and therefore can be utilized economically. A combination of reagents can be used. For example, proteins can be selectively precipitated with PEG followed by addition of tannic acid which interacts with the PEG, thereby liberating the protein in solution.

### 3. Selective Inactivation by Inhibition

Various reagents are available for the inactivation of specific enzymes. For example, sulfhydryl-blocking agents are capable of specifically inhibiting enzymes that require sulfhydryl groups for their activity. Also, enzymes that require divalent metal ions like $Ca^{2+}$ and $Mg^{2+}$ for activity can be inactivated by chelating agents like EDTA, EGTA, etc.

4. Genetic Methods

It may be possible to use genetic engineering techniques to remove undesirable enzyme activities or introduce/enhance desirable enzyme activities. For example, by the use of deletion or temperature-sensitive mutations one could conceivably generate cellulase$^-$ or protease$^-$ mutants of the fungus (*A. terreus*) that could be useful at different stages of jute processing.

For the last several years such mutation has been tried at IJIRA with conventional methods, namely, ultraviolet light, nitrosoguanidine, alkylating agents, ethidium bromide, and other mutagenic reagents. However, such conventional methods have not met with success thus far at IJIRA. It is possible that repair enzymes are quite potent in these systems, thereby negating the mutations. A role for modern genetic engineering (site-directed mutagenesis or protein engineering) can be envisioned here. Unfortunately, not enough genetics is available for this particular fungal strain (*A. terreus*). Some work was initiated by Dr. Sandip Sinha during his fellowship tenure at Oak Ridge National Laboratory, particularly genome isolation and its size determination. The next step is the cloning of the fungal enzymes for which the development of appropriate cloning vectors is urgently needed. Once cloned, the enzyme activity could be manipulated through the use of protein engineering.

## B. Progress in Downstream Processing

One of the first steps in the use of enzymes in the jute industry involves aqueous extraction of dry moldy bran which contains the extracellular enzymes produced by the fungus *A. terreus*. For the users (the jute mills) this represents an additional step prior to the applications of the enzymes.

Ultrafiltration as a downstream processing step has been used extensively for purification and concentration of enzymes and proteins. This methodology was explored by Dr. S. N. Sinha of IJIRA during a work assignment at Pennsylvania State University, U.S.A., using first a 30,000 m.w. cut-off membrane followed by a 10,000 m.w. cut-off membrane. The recovery of cellulase in the retentate

fraction using the 10,000 m.w. cut-off membrane was about 73% and over 5-fold concentrated, whereas the recovery of alkyline protease was poor. On the other hand, with the use of a 30,000 m.w. cut-off membrane the alkyline protease recovery was about 37% and about 3-fold concentrated. Thus, it appears that fractions enriched in cellulase (for jute fibre softening) and those enriched in protease (for Biomodification of TKP) can be obtained by ultrafiltration. Further work is required to increase the recovery of protein.

In view of the above, it is of great importance to purchase an ultrafiltration setup along with a continuous flow centrifuge. This equipment should be established at IJIRA since considerable research will still need to be done to optimize the downstream processing. Basically, two types of enriched and concentrated enzyme fractions will be produced: one will be useful for fibre softening while the other will be used for biomodification of TKP size. Both processes will be highly beneficial to the jute industry.

## IV. VISITS AND INTERACTIONS

### A. Visits to Jute Mills

I visited three jute mills where I was shown every detail of jute processing from receipt of raw jute to packaging of finished material to production of jute fabrics and carpets.

The first visit was to Kinnison Jute Mill where I was shown the Enzyme Plant, designed for IJIRA by Central Food Technological Research Institute (CFTRI) at Mysore. Details of the project can be obtained from the report entitled "Detailed Project Report on Establishment of Solid State Fermentation Culture Plant for Production of Enzyme Complex for Use in Jute Industry" (July 1988). The Enzyme Plant is almost reaching completion. My guide, Dr. S. N. Sinha, Coordinator of the project, thoroughly explained every detail of the process. This plant will produce the enzyme extract from moldy wheat bran for use in the biosoftening of jute. Biomodification of tamarind kernel powder (TKP) by the enzyme extract from moldy bran will also be carried out. Enzyme-treated TKP

will be applied directly to jute fibres for improved sizing. This plant will serve as a supplier of enzyme extract to various jute mills both for biosoftening of jute and for improved sizing.

As discussed in the previous section, ultrafiltration promises to be a very useful technique for obtaining fractions enriched in cellulase for jute fibre softening and those enriched in protease for the biomodification of TKP for jute sizing. Therefore it makes sense to also set up an ultrafiltration unit at the Enzyme Plant at Kinnison Jute Mill. The ultrafiltration unit proposed to be set up at IJIRA could be used for further methodological developments.

My next visit was to the India Jute Mill. This is an excellently run operation under the superb management of the General Manager, Mr. J. Sharma. I was shown every detail of the jute processing and the uses of the enzyme extract and biomodified TKP. Mr. Sharma and the jute mill personnel seemed very happy about the use of these biomaterials. Problems that occasionally crop up are immediately brought to the attention of Dr. S. N. Sinha and solutions are then devised in consultation with the IJIRA research staff. This is an excellent example of transfer of basic science and technology from the public to the private sector. For example, during this visit we discussed the possibility of using inhibitors to prevent occasional microbial infection of jute, particularly during the rainy season.

At India Jute Mill, I was quite impressed with the quality and design of rugs and carpets being made from jute. The application of enzymes to the jute certainly added lustre to the jute being used to make the carpets. I believe jute carpets will capture an increasing share of the market in the near future.

My next visit was to the Hastings Jute Mill where we were shown around by Mr. R. K. Mahajan, General Manager. Here also the applications of enzyme treatment and biomodification have made their mark. These treatments have enhanced the upgrading of very coarse jute fibres, which used to be discarded not too long ago.

B. Visits to Libraries for Literature Search on Downstream Processing

The usual methods of selective protein precipitation and purification carried out in research laboratories often need to give way to more economical and large scale methods in industry. Before leaving for my assignment I had done a library computer search for improved protein purification methods used in industry and collected a long list of such articles. On arrival at IJIRA I visited several local libraries to locate some of the journals containing these articles. Some were located at IJIRA and others at the Indian Institute of Chemical Biology in Calcutta.

I plan to continue the search in the USA and, if necessary, mail copies of the articles back to IJIRA.

C. Interactions with Key Personnel in the UNDP Project -- Ongoing and Future Research Plans

This was perhaps the most useful and enjoyable part of my visit. The key people involved in the project are

Dr. B. L. Ghosh, Head

Biology Division, IJIRA

Dr. S. N. Sinha, Coordinator

UNDP Project, IJIRA

Dr. S. K. Chakrabarti, Staff Member

Biology Division, IJIRA

Mr. D. Ghosh, Junior Member

Biology Division, IJIRA

Dr. S. N. Sinha, as Coordinator of the Project, was the person with whom I interacted on a daily basis. We met regularly for discussion of past work, visits to the biological laboratories and the pilot plant at IJIRA, visits to the jute mills, and planning for future work.

With Dr. Sinha, I also met with Drs. B. L. Ghosh and S. K. Chakrabarti several times to discuss ongoing research and future plans as follows:

Application of biochemical techniques, besides future use of genetic engineering, for improving the inactivation of cellulases in the enzyme preparation produced during biofermentation in bran culture. It should be pointed out that besides proteases, the extract from moldy bran contains cellulases and hemicellulases. Although cellulases are important for softening of jute, they are deleterious during the biomodification of TKP for application to jute fibres. At the present time, inactivation is done by heating, which also partially inactivates the proteases and is expensive on an industrial scale. We discussed the possibility of using selective inhibitors. For example, we already know from the work of Dr. S. K. Chakrabarti while at the Department of Microbiology, Colorado State University (see Report of Dr. S. K. Chakrabarti, July 1990) about the biochemical properties of the purified protease activity. Similar studies need to be done with the cellulase activity. For example, the protease is activated by several metal ions, namely, $Mn^{2+}$, $Cu^{+}$, $Ca^{2+}$. It would be interesting to test these metal ions to see whether they would inactivate the cellulase activity.

I suggested the use of biochemical methods for the possible separation of the protease and cellulase activities. As discussed in a previous section, selective precipitation methods may be quite useful. Under the guidance of Drs. S. N. Sinha and B. L. Ghosh, Dr. Chakrabarti is now testing several reagents, for example, polyethylene glycol, tannic acid, polyacrylic acid, etc. The main advantage of these reagents is that these are byproducts of other industries, and therefore quite economical.

We also discussed the possibility of physical separation utilizing ultrafiltration, which has wide-spread applications in industry. With the hope of setting up an ultrafiltration unit at IJIRA, this becomes an attractive possibility.

Discussion also centred on the importance of applying modern molecular biology and genetic engineering to the biomodification process. As discussed before, this would need further fellowship training of key personnel from IJIRA in foreign laboratories, followed by obtaining suitable grants from the Government of India to pursue the projects at IJIRA with graduate students and young investigators.

I had extensive discussions with Drs. B. L. Ghosh, S. K. Chakrabarti, and S. N. Sinha regarding the bleaching and increased brightness of jute fabric by the application of the enzyme extract from moldy wheat bran. Preliminary evidence in the laboratory suggested that the observed changes may be due to metal-chelating agents in the enzyme preparation. Further research is necessary to identify the beneficial agent(s). Once identified, such agents can possibly be directly applied to jute fabric.

## V. MY CONTRIBUTIONS TO THE PROJECT

1. Providing the facilities and training of the technology of protein engineering during the UNDP fellowship tenure of Dr. S. N. Sinha in my laboratory. Since protein engineering is a marriage between two fields -- molecular biology and biochemistry -- it requires the applications of diverse techniques. Dr. Sinha learned a lot of these techniques during his fellowship tenure.
2. Providing the laboratory setup for preliminary work on the DNA from the fungus *A. terreus*. The DNA was isolated and preliminary characterization was done.

3. Bringing new fungal and bacterial strains from American Type Culture Collection to IJIRA to be tested for possible use in the biomodification process.

4. Computer search of bibliography relating to downstream processing, with special reference to novel and economical methods of selective protein precipitation to separate the protease and cellulase activities in the moldy bran extract. Library search was continued by me after arrival at IJIRA.

5. Planning of protein precipitation work with Drs. S. N. Sinha and S. K. Chakrabarti. The studies have now been initiated at IJIRA.

6. Imparting computer know-how for use in high performance liquid chromatography (HPLC), and other modern laboratory procedures.

## VI. RECOMMENDATIONS

In view of the success of biosoftening and biomodification in the jute industry, it is highly important to continue this project. The specific recommendations that could possibly benefit the program are as follows:

1. Development of traditional biochemical approaches for separation of the cellulase and protease activities in the moldy wheat bran extract should continue at a faster pace. Possible methods have been discussed extensively in this report.

2. The development of modern molecular biology should be pursued at IJIRA. Genetic engineering techniques might be employed to either remove an undesirable enzyme activity or to introduce/enhance a desirable enzyme activity at a particular step of jute processing. As discussed earlier in this report, the genetics of *A. terreus*, the fungal strain used at IJIRA, needs to be developed. The cloning of the appropriate cellulase and protease activities can then follow. Once cloned, modern techniques of protein engineering can be employed.

3. It is of the utmost importance for project staff at IJIRA to gain further expertise in modern molecular biology through UNIDO fellowship training. The training period should be extended to at least one year, rather than the customary six months, to achieve meaningful results.

4. Upon return from fellowship training, project staff should be urged to apply for extramural research funding from the appropriate granting agencies of the Government of India. Utilizing such funds University (Calcutta and Jadavpur being examples) students should be recruited to do their thesis or postdoctoral research at IJIRA. This could lead to the infusion of new young talents to IJIRA, which is of great importance for the successful development of the institution.

5. Technology transfer -- the flow of ideas stemming from basic research in the laboratory leading to applications in the industrial sector, as exemplified in the interactions of IJIRA scientists with jute mill personnel -- should continue to be encouraged by IJIRA management. In fact, this activity should be expanded.

In conclusion, it is quite clear that the applications of enzymes have already proved to be quite beneficial to the jute industry. The research staff has made significant contributions utilizing traditional methods of enzymology. Further development of such methods along with the applications of modern molecular biology should continue. The support of UNIDO has been highly important for the Project and continued support is indispensable for the Project's future.