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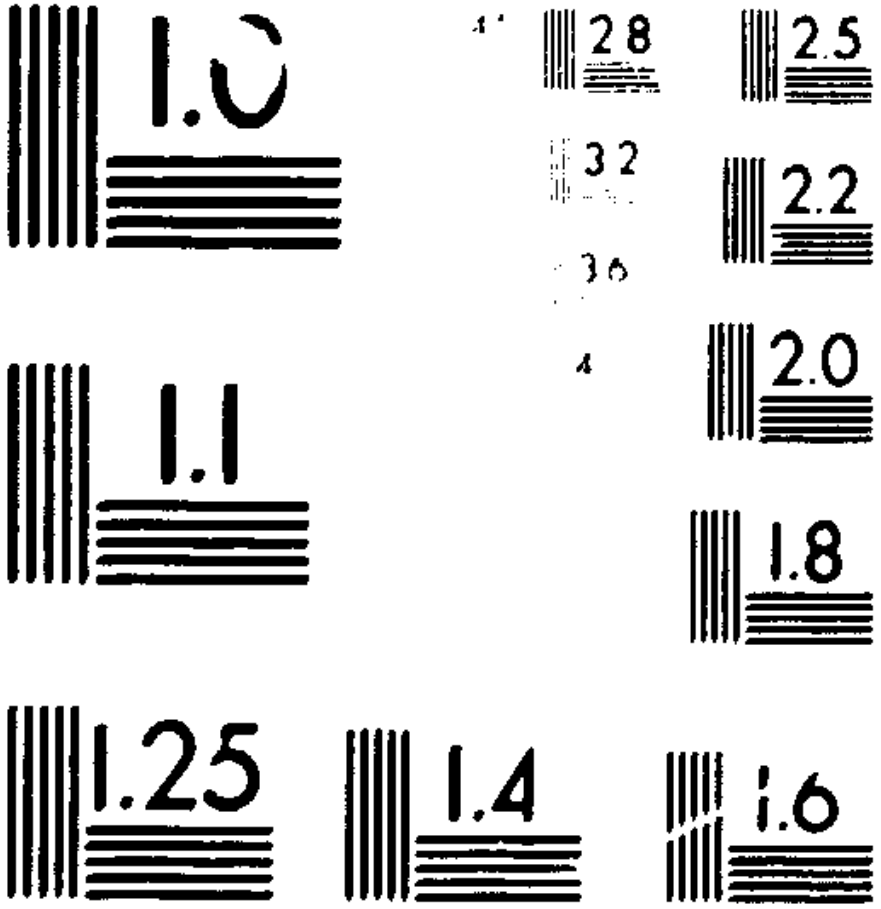

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DP/ID/SER.A/576
29 March 1985
ENGLISH

JUTE PRODUCTS RESEARCH

DP/BGD/75/013

BANGLADESH

Technical report: Application of biotechnology to the softening of jute cuttings *

Prepared for the Government of Bangladesh
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of J. A. Green,
Consultant in Microbiology

United Nations Industrial Development Organization
Vienna

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ACKNOWLEDGEMENT

The Consultant wishes to thank Dr. G. Mohiuddin and the Staff of his Department for their interest and co-operation during this mission, all staff at BJRI for their friendliness and warm hospitality, and the UNIDO/UNDP Staff, Dr. A.M. Bhuiyan, Director and Dr. Md. Ayubur Rahman, Director General BJRI for their co-operation.

MISSION SUMMARY

The Consultant visited BJRI during October-December 1984, and the results of the mission are summarised below:

1. A programme was formulated for developing an improved process of softening jute cuttings by biotechnological means. The most favoured approach would be to alter the environment in mill piling and promote softening while retaining current technology. Failing this, SSF could be investigated using bark degrading microorganisms and their mutants and could possibly be applied in mills as a low-technology process. Alternatively enzymes capable of bark-degradation could be applied to softening. This would probably be the most controllable process, but would involve establishing enzyme production units in mills and would perhaps be least acceptable to mill operators.

2. The following techniques necessary to carry out the programme of development were demonstrated and developed during the mission:- General microbiological techniques; Laboratory-scale fermentations; Isolation of bark-degrading organisms; Simple screening procedures for relevant enzyme activity; Production of mutants; SSF; Enumeration of selected organisms during the piling process.

INTRODUCTION

The purpose of this mission was to assist BJRI (Tech Res Wing) to develop its microbiological research programme especially in the areas of nutritional problems associated with the growth of anaerobic bacteria (see Appendix 1). The areas identified as important were:

(1) To take part in research work concerned with the removal of non-fibrous material from inadequately retted jute fibre.

(2) To advise particularly on provision of nutrients to give more profuse growth of bacteria associated with retting.

Prior to commencing this mission the Consultant held a briefing in Edinburgh with Dr. H.P. Stout, who provided an outline of the objective of the mission and general background information on BJRI and UNIDO's assistance to it. During this meeting Dr. Stout identified area 1 above as being of great importance to the jute industry of Bangladesh, and the Study Tour Report by Dr. Ghulam Mohiuddin (supplied by Dr. Stout) reaffirmed this.

The Consultant left Edinburgh on 25 September 1984, was briefed in Vienna and commenced activities at BJRI on 1 October 1984. Activities at BJRI ceased on 19 December 1984 and a debriefing was held in Vienna and with Dr. Stout in Edinburgh.

After commencement of the mission and discussion with Dr. Ghulam Mohiuddin at BJRI the objectives were revised into a long-term objective of developing a biotechnological process for the removal of bark from jute cuttings (i.e. Area (1) above) and an immediate objective which was to demonstrate and develop the techniques which would be required to carry out the long-term objective.

This report consists of details of the problem of softening jute cuttings, the activities of the Consultant during the mission and proposals for a programme of research into biotechnological softening of jute cuttings.

I. SOFTENING OF JUTE CUTTINGS

After retting and separation of fibres the bundles of jute fibres are transported to mills for processing. According to the variety of plant from which the fibre derives and the growth conditions a proportion of the fibres from the base stem have unretted bark material adhering to them, which makes fibre separation without further processing difficult or impossible. If the farmers prolong the retting period to complete the removal of bark from the lower stem portion then the fibres in the upper stem portion are over-retted and suffer degradation and loss of quality.

The conventional method used by the industry for the removal unretted bark meterial involves cutting off the portion of the fibre bundles containing adherent bark (the resultant 'cuttings' comprising anything from 5-25% of the total fibre length). The cuttings are sprayed with a mineral oil-water emulsion to CA 30% moisture content followed by piling in large brick or concrete bins (some 15 ft.X15ft.X15ft. or 5mX5mX5m). This represents some 3-4 tons of cuttings. The bins may be covered by sheeting and are left for up to 15 days, during which time the temperature in the bins rises to CA 70°C. It is not known whether direct microbial action produces the resultant softening and at BJRI the assumption is that the sustained high temperature causes permeation of water into the bark and produces softening in this way. Microbiologically the process is composting, where thermophilic anaerobic or facultative anaerobic microorganisms predominate, and cause the temperature to rise as a result of exothermic metabolic reactions. In the past a supplement of urea (nitrogen) has been added to the mineral oil emulsion to stimulate microbial activity and softening, but this practice has been discontinued becuase mill workers complained of adverse reactions from handling the field cuttings (presumably from ammonia produced during microbial degradation of urea).

Once cuttings have been composted in this way the bark is only partly softened and the recovered fibre is coarse and uneven with specks of bark remaining, causing difficulties in spinning and weaving. Thus the biotechnological removal of bark fro. ttings would be of considerable

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assistance to the Bangladesh Jute Industry by allowing easier handling and effecting upgrading of fibre quality.

Biotechnological solutions previously attempted

A group of workers at IJIRA have published a biotechnological method by which they claim that jute fibre can be upgraded (1) and jute cuttings softened (2). The procedure involved the cultivation of the fungus Aspergillus Terreus in solid-state fermentation (SSF) on wheat bran, extraction of a crude mixed-enzyme preparation from the bran and incorporation of this preparation into the oil emulsion applied to cuttings prior to composting. It was claimed that such treatment improved the softening of cuttings, and upgraded the fibre leading to a net cost saving. It is not known how widely practiced this procedure is in the Indian Jute Industry or how effective it is in practice.

The group at IJIRA assumed that the softening effect was due to a synergistic effect of the enzymes and the natural bacteria, although the possibility of an effect from the inadvertent addition of nutrients, organic acids and fungal inoculum was not considered. Such treatments have not been applied in the jute industry in Bangladesh as pilot scale studies at BJRI did not show the treatment to be effective. (However see section I, 1).

II. LONG-TERM OBJECTIVE

The long term objective of this mission was to assist BJRI with the development of an applicable biotechnological process for the improved softening of jute cuttings.

The other objective set by UNIDO (viz. to advise on the provision of nutrients to give more profuse growth of bacteria associated with retting) was considered not pertinent to problems faced by the jute industry.

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Programme of research and development

The most acceptable solution to the softening problem to the jute industry would be to modify conditions during composting while retaining current technology. Failing this, a modification of existing technology might be possible using SSF or the production of specific enzymes for application to cuttings.

(1) Modification of composting conditions using current technology.

Modification of composting (piling) conditions could be varied empirically in several ways, and the effects on softening noted. The factors which could be varied and the rationale for selecting them are given below:

(i) Moisture content and salt concentration. The moisture content of cuttings will affect the water activity (AW) in the growth environment and will influence which microorganisms in the environment will predominate. Altering the moisture content could affect microbial softening of the cuttings. The addition of salt likewise will affect the Aw of the growth environment and could influence microbial activity. Addition of an increased moisture content in conjunction with a high salt concentration could give an Aw (and microbial activity) equivalent to low moisture content aline, while perhaps giving an increased moisture content for improved physical softening (if indeed a physical effect is involved in softening).

(ii) Nitrogen concentration. It is already known that a nitrogen supplement has a stimulatory effect on microbial growth and activity during piling (see section I). The possible deleterious effect of ammonia production from urea might be avoided using lower concentrations of urea, substituting ammonium salts for urea, or using urea in combination with other treatments (such as altered Aw, or level of phosphate carbonate or citric acid).

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(iii) Phosphate concentration. The supplementation of cuttings with phosphate would have a stimulatory effect upon microbial growth and activity (particularly if given in combination with nitrogen). Phosphate addition would also tend to buffer the pH and could also influence microbial activity in this way.

(iv) pH. The addition of organic acids (such as citric acid) may lower the pH and stimulate the growth of organisms (such as actinomycetes) involved in composting (which are acid producing and thus have growth optima at low pH). This treatment in combination with nitrogen-supplementation might overcome the problem of ammonia production. The addition of carbonate would leap to an increase in pH.

(v) Combined treatment. It would probably be worthwhile to look at the influence of factors (1)-(iv) in combination as combined treatments may have beneficial effects (in the manner described for some combinations above).

These experiments could be carried out on the laboratory-scale, or on pilot-scale using the piling bins at BJRI. However neither of these situations mimics the rise in temperature experienced in piling in the mills (they are mesophilic processes and perhaps aerobic, as opposed to the thermophilic anaerobic process of composting).

As it would not be practicable to carry out these studies on the entire contents of mill bins, a solution might be to carry out experiments on small batches of cuttings placed in a box or tray which itself would be placed in the centre of a production bin. In this way laboratory or pilot scale experiments could be conducted in an environment (with respect to atmosphere and temperature) representative of mill conditions (although this assumes that the treatments being studied would not alter the temperature if applied to the contents of the entire bin !) Alternatively it might be possible to mimic bin conditions in the laboratory by composting in sealed vessels (to ensure an anaerobic environment) incubated at

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bin temperatures (to ensure selection of thermophilic organisms).

(2) (i) SSF. SSF has recently been extensively studied in many countries, particularly with a view to prouce SCP from solid wastes. It has also been applied to the pre-treatment of lignocellulosic material for pulping in paper manufacture (3). Although there has been development of high-technology processes (4), SSF can be carried out using low-technology processes (as is the case with the Koji process (A traditional SSF used extensively in Asia, where layers of rice inoculated with fungal spores are spread out in woven bamboo trays) and citric acid production. The distinction (in microbiological terms) between SSF and composting is that the former is a mesophilic aerobic process (the term fermentation is a misnomer) while the latter is a thermophilic anaerobic one, while both involve the growth of microorganisms on a solid natural substrate.

SSF could be applied to softening jute cuttings as a low-technology process for the removal of bark substances. Laboratory scale experiments could be performed under aseptic conditions using cuttings inoculated with pectinolytic and/or cellulolytic and/or ligninolytic organisms (including mutants) and the effect of environmental conditions (such as those outlined in Section II (1) p) on the process studied. Pilot scale experiments in unsterile conditions (which would obvious be more acceptable to mill operators) could be performed using the pilot-scale bins at BJRI. The development of a working process (i.e. unsterile) from laboratory-scale experiments with pure cultures would pose many problems, but it might be possible to adjust the enviromental conditions to stimulate the growth of the inoculated organism while inhibiting competitors (e.g. using a high salt concentration or low pH (by citric acid addition) to selectively cultivate a fungus.

Should a process of softening be developed using SSF, the process (being one of low-technology) should be acceptable to the Industry,

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now and in the future. SSF to soften cuttings could be conducted in existing bins by constructing slatted shelves within the bins at, say, 18-24 inch spacing, and layering cuttings on these shelves to a depth of, say, 9-12 inches. Development work would be needed to determine the optimum layer depth and shelf spacing to ensure the maintenance of aerobic conditions. Alternatively the cuttings could perhaps simply be spread out on a floor (or the ground) and occasionally 'Turned' (Turned-over) by hand to ensure aerobic conditions are maintained.

(ii) Enzyme technology. IF a solution to the softening problem cannot be developed along the lines outlined above, it might be possible to produce softening by the application of microbial enzymes. This could be carried either by a low-technology process such as adopted by the workers at IJIRA (i.e. application of crude enzymes produced by SSF - see section I), or by a high technology process of enzyme production by fermentation (although this technology is probably not appropriate to Bangladesh at present.)

Pectinase, cellulase or hemicellulase enzymes could be produced by fermentation and their softening (singly and in combination) effect noted, which would at least indicate which activity (or combination of activities) was responsible for the softening effect. Studies at BJRI indicate that pectinase activity is likely to promote softening of cuttings and so would be the logical starting point. However, ligninase cannot easily be produced by fermentation as it is intracellular and would require elaborate preparative techniques (and so SSF remains the best option for lignin removal).

The advantage of the use of enzymes over SSF is that they could be applied evenly to cuttings for treatment (and hence the activity would be evenly distributed) whereas in SSF the growth and activity of the microorganisms could be patchy. However enzyme production would involve the establishment of production centres in mills, whereas SSF could perhaps be developed without the need for specialized sections within the mills.

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3. Test for softening The work published by the team so far on bacterial softening of cuttings has used a subjective visual and tactile assessment of the degree of softening achieved. While this might be satisfactory for laboratory studies, for the development programme outlined above it would be desirable to back this up with a more meaningful quantitative (or semi-quantitative) measurement of the degree of softening achieved, using physical measurements and spinning quality of the fibre. The team has experience of such measurements and should apply them wherever possible in the development programme.

III. SHORT-TERM OBJECTIVE

A. Demonstration and development of techniques

The objective for the duration of this mission was to demonstrate and develop the techniques required to carry out the long-term objective. These techniques were as follows:

(1) General microbiological techniques, particularly the isolation and maintenance of pure cultures and the manipulation of fungi.

(2) The isolation of microorganisms capable of degrading the bark of jute cuttings.

(3) The isolation of microorganisms capable of degrading the bark of jute cuttings.

(4) Screening procedures to detech pectinolytic, ligninolytic and cellulolytic activity in microorganisms.

(5) The production of mutant microorganisms using UV radiation.

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(6) The technique of laboratory-scale SSF.

(7) The study of the microbiology of the current piling (softening) procedure.

B. Rationale for selecting techniques and technical details

The rationale for selecting the techniques outlined above and some technical details are given below.

(1) General Technique. Various microbiological techniques, especially the manipulation of fungi, were demonstrated to team members. One of the team-members, Mr. Abul Kashem, has applied for a 6-month traineeship, and the consultant strongly recommends approval of this and further recommends that in this and any future traineeships as far as possible the opportunity is taken to gain experience of microbiological techniques.

(2) Laboratory-scale fermentations. A laboratory-scale fermentation unit had been supplied to the Microbiology Department at BFRI (partly with UNIDO funding) and the consultant assembled this unit and demonstrated its use for batch and continuous (Chemostat) cultures, and a schedule of the procedure was produced. (Appendix 2).

This equipment will be valuable to the Department for (1) the production of enzymes of microbial cells (biomass) on a comparatively large scale for application to cuttings (and in the chemostat mode produced under controlled and reproducible conditions) and (2) the study of the effect of environmental conditions (in the chemostat mode) on enzyme production or other physiological characteristics.

(3) Isolation of bark-degrading organisms. In the programme to develop a process for the removal of bark from cuttings microorganisms (or their enzymes) capable of degrading bark components (lignin, cellulose, hemicellulose, pectin and waxes) might be required. Such organisms might

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be available from the culture collection at BJRI or laboratories elsewhere or they could be isolated from naturally degrading jute cutting bark or from enrichment cutures. The technique of enrichment culture was demonstrated and a schedule of the procedure produced (Appendix 3), and fungi were isolated from these and from naturally degrading cutings.

A stock of bark degrading organisms should be established and maintained by the Department, for use in the development programme.

(4) Screening for relevent enzyme activity. It was likely that organisms isolated from the procedures outlined in(3) above would have a spectrum of enzyme activity, for instance the ability to degrade lignin is usually coupled with the ability to degrade cellulose. However, organisms such as 'Cellulaseless Mutants' could be extremely useful in this project (By degrading bark lignin without removal of the strength-giving cellulose in the fibres). The study tour report of Dr. Mohiuddin outlined the need for a simple screening procedure for ligninase, cellulase and pectinase activity and after reading the report prior to the mission the Consultant carried out a literature survey prior to the mission and communicated information on the techniques to the team. The methods for cellulase and dectinase were tested and developed during the mission.

(5) Production of Mutants. As outlined in 3) and 4) above, it was likely that mutant microorganisms with an altered pattern of activity (such as cellulaseless ligninolytic organisms or high yielding strains) would be of use in this project. UV radiation is a convenient and safe method of inducing mutation in microorganisms, and this technique was demonstrated and a schedule of the procedure produced (Appendix 4). This work was hampered by lack of controlled incubation conditions and contamination of plates.

(6) Solid substrate fermentation. SSF is being applied in several fields, and in particular to the production of SCP and chemicals from lignocellulosic agrowastes. It could be an appropriate low-technology process for

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the removal of bark from cuttings by treatment with aerobic organisms (such as pectinase and/or ligninase and/or cellulase producers). Literature on the theory and practice of SSF on a large-scale was supplied to the team and the technique was demonstrated on a laboratory scale and a schedule of the procedure produced (Appendix 5).

The technique could also be applied to other problems of interest to BIRI viz. the utilisation of industrial and agricultural wastes.

(7) Microbiology of the present piling process. The initial approach to solving the softening problem will be to adapt the current composting procedure (see Section II (1)). Thus some physical and microbiological base-line data on the current composting process would be useful and would also encourage the team to pay regular visits to the mills and acquaint themselves closely with current technology.

Useful physical measurements would be temperature, pH and moisture content and useful microbiological measurement would be microbial loading (Total and selected groups). The rapid indirect method of methylene blue reduction for total microbial loading was attempted but was not sucessful. Other indirect methods such as phosphatase or ATP assay were not attempted, being too involved and expensive. The method of direct counting after standardised washing of cuttings was adopted and a schedule on the procedure produced (Appendix 6).

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RECOMMENDATIONS

A. Equipment

The Department lacked the following items of basic equipment, which would be necessary to carry out the development programme:

1) pH Meter
2) Autoclave
3) Freeze-Dryer (Requested in 1980 !)
4) Adequate supply of Pipettes

Facilities for aseptic work (such as plate-pouring and inoculation) were not ideal, and the atmosphere in the laboratory was heavily contaminated. A working cabinet is to be constructed with an internal UV lamp for sterilisation, but should this fail to provide an adequate environment for aseptic manipulation the purchase of a laminar-flow cabinet must be given serious consideration.

The 5 litre fermentation unit, parts of which have already been supplied, should be completed (By purchase of the vessel and a pH control unit) so that reasonably large batch fermentations can be carried out.

For the isolation and enumeration of microorganisms and mutation experiments it will be essential to grow organisms at controlled constrant temperatures below ambient (Even during the winter period). The Consultant strongly recommends purchase of a controlled temperature (i.e.Heating/Cooling) incubator for this purpose.

B. Experience and Traineeships

It was apparent that experience of microbiological techniques needed strengthening in junior members of the department. Opportunity should be

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taken during traineeships to gain such experience (and the department should ensure that such experience is fully utilised - see Recommendation D), and any future recruitment should if possible be of personnel with some microbiological experience.

During the mission approval was granted by Napier College, Edinburgh, for trainees to spend training periods with the Consultant, who would design an programme to give appropriate microbiological experience. BJRI (Tech Res Wing) may wish to take advangage of this.

C. Biotechnological Development at the Institute

The Consultant considers that biotechnology can have a role in jute processing (Especially in the long-term) and regrets that assistance in the form of consultancy has been rendered to BJRI (Tech Res Wing) so late in the UNIDO Assistance scheme. BJRI is urged to recognise the importance to the Industry of improved softening of cuttings by biotechnological means and to ensure that this is given appropriate priority in allocation of resources. The advantage of biotechnological treatment over chemical softening (As suggested by K.V. Sarkanen - Final Technical Report, Section IV B) is that the former (unlike the latter) would not involve a high energy (Heat) input and might not depend on imported chemicals.

It has been pointed out (Study Tour Report of Dr. Maniuddin) that biotechnology could be applied to the utilisation of jute wastes, although a previous consultant K.V. Sarkanen was dismissive of this notion (Final Technical Report, Section IV. F) and suggested chemical processing. The Consultant considers biotechnological conversion of jute wastes to merit investigation, and such technology could be applied more widely in the future to other agrowastes.

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D. The Approach to Research and Development

Within the Institute it appears that particular personnel are often asigned to particular projects. The disadvantages the Consultant sees in this are as follows:

1) It can lead to lack of flexibility of approach (Someone will be reluctant to change topic or emphasis while half-way through a Ph.D. project).

2) It means that it is difficult to stimulate team effort and effectively utilise (within a Dept. as a whole) skills gained on traineeships or elsewhere. BJRI should consider the disadvantages outlined above and ensure a flexible approach to development based on teamwork and a pooling of expertise.

E. Relationship Between the Institute and the Jute Industry

The Consultant endorses the view of a previous Consultant, K.V. Sarkanen, that 'since the principal function of the Institute is to develop new improved technology for jute mills, more intensive interaction with the Jute Industry is desirable, particularly in the formation of its research (and development ?) programme'. One way of promoting this might be to increase membership of the Research Committee from representatives of the Industry and to make this Committee a more effective review and consultative body. A change in title from BJRI to Bangladesh Jute Technology Development Centre might also promote a change in emphasis to technological development.

F. Future Directions of the Institute

The Consultant also endorses the view of K.V. Sarkanen that the Institute should give increased attention to implementing processes in the Industry and that UNIDO assistance with this would be of great benefit.

UNITED NATIONS

APPENDIX 1

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

UNIDO 18 June 1982

PROJECT IN THE PEOPLE'S REPUBLIC OF BANGLADESH

INTERNAL

JOB DESCRIPTION

DP/BGD/75/013/11-51/31.7.B

Post title	Microbiologist
Duration	Three months
Date required	As soon as possible
Duty station	Dacca
Purpose of project	To assist the Bangladesh Jute Research Institute (Tech) to develop its microbiological research programme especially in the areas of nutritional problems associated with the growth of anaerobic bacteria.
Duties	The consultant will specifically be expected to: 1. Take part in the research work concerned with the removal of non-fibrous material from inadequately retted jute fibre; 2. Advise particularly on the provision of nutrients to give more profuse growth of bacteria associated with retting. The consultant will also be expected to prepare a final report, setting out the findings of the mission and recommendations to the Government on further action which might be taken.
Qualifications	Post-graduate degree in Microbiology/Bacteriology; considerable experience in the study of nutritional aspects of anaerobic and aerobic bacteria, or bacteria of industrial importance.
Language	English

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Applications and communications regarding this Job Description should be sent to:

Background Information

Jute is the most important export commodity of the country and also the raw material for the country's most important industry. The jute industry was completely nationalised in 1972 and has been making steady progress since then towards achieving the 1969-1970 production level, which was the highest in its history.

There are currently 77 jute mills in the country, of which 74 are in operation. The estimated production in 1974-1975 was 451,000 metric tons of jute fabric in the form of hessian, sacking and small quantities of carpet backing; this represents approximately 50% of actual installed capacity. Approximately 85% of this production goes to the export market, earning 55% of the country's foreign exchange. The industry provided employment for 200,000 people.

In the last ten years, and particularly in the early 1970s, jute has been subject to increased competition from synthetics, mainly from polypropylene. The successful market development of polypropylene in direct competition with jute can be attributed to its low price, ready availability and superior technical performance as a result of superior mechanical and physical properties. A typical example of the prevailing situation is in the area of primary carpet backing in the US market, where the share of jute has fallen from over 80% in 1967 to less than 30% in 1974. The decline in other markets and other traditional jute end-users has been equally precipitous.

While in the short term a policy of containment to minimise market losses on the part of the jute producing countries must necessarily focus on the price imbalance between jute and synthetic products, the survival of the jute industry will depend on the increase of its productivity and the development, through research, of better products and new end-users. This project has been designed with the latter aim in mind.

The Dacca Declaration of January 1973 envisaged the establishment of Jute International with a mandate to carry out joint research and development activities. UNDP subsequently fielded a Research and Development Working Group to recommend priority projects to Jute International. The technical centre envisaged to be established under Jute International would be fed with results from various national and international reserach centres for effective translation as and when necessary. The Research and Development Working Group also stressed the need for viable national research centres to carry out their own research projects which will be valuable to their own national industries.

In the country, the mandate for carrying out technological research has been given to the Bangladesh Jute Research Institute. The Institute consists of an agricultural research wing and a technological research centre. Facilities at the technological research centre are inadequate to carry out any meaningful research and development project. In addition, the centre is understaffed and lacks expertise in all the important areas of jute technology. As the report of the research and development working group for Jute International states, assistance to the Bangladesh Jute Research Institute is urgently needed to improve its own facilities for research and development, irrespective of related technical activities planned for Jute International.

ANNEX 2

OPERATION OF THE MODULAR FERMENTER (BATCH FERMENTATION)

INOCULUM PREPARATION

(1) Bacterial	Day Minus 2	Prepare a staeak plate for isolated single colonies from a refrigerated stock culture.
	Day Minus 1	Inoculate a nutrient broth culture in a univeasal bottle from a single colony from the plate prepared on day - 2.
(2) Fungal	Day Minus 1	Prepare a streak plate for isolated single colonies from a refrigerated stock culture. Incubate until isolated colonies develop.
	Day ?	When isolated colonies have developed inoculate a potato-dextrose agar slant from a single colony. Incubate until the growth on the slant appears fluffy.
	Day 1	Flood the slant with sterile medium/water containing 0.1% tween 80 (detergent). Use a sterile loop to suspend the fungal growth/ spores in the medium/water and aseptically transfer the suspension to a sterile universal. Use this as the inoculum.

FERMENTER PREPARATION

Day Minus 1	(1)	Clean and wash the vessel interior and all tubes containing liquids.
	(2)	Calibrate the pH electrode against a standard buffer solution (preferably pH for use with bacteria and pH 4-5 for use with fungi). Check the level of electrolyte and top-up if necessary.
	(3)	Prepare 500 ml medium and place in the vessel (if glucose or other carbohydrate is included make this up in 10-15 ml medium in a universal and autoclave separately).
	(4)	Top up the antofoam, acid and alkali reservoirs. (Use 0.5M acid/alkali and 10 ml antifoam in 200 ml water).

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(5) Secure the top-plate, all tubes, bottle-tops and clips and check that all the gland nuts on the top-plate are tight.

(6) Autoclave at 10 PSI for 25 mins.

FERMENTER OPERATION

Day 1

(1) Connect all the tubes to the apropriate pumps, replace the electrode leads and connect the water supply. Clamp the vessel to its stand.

(2) Inoculate the medium (and add the glucose if autoclaved separately) using the inoculation system.

(3) Check the purity of the inoculum by

(i) Staining

(ii) Preparing a streak plate for isolated colonies. (Decant a few ml culture into a sterile universal using the sampling system.)

(4) Adjust the stirrer speed (Mark 4-5), temperature control (for cooling only set the temperature to 20°C), Air Flow (100-200 cc/min) and antifoam addition (set to 10 secs delivery/hour).

ON SUBSEQUENT DAYS

(1) Check the culture purity by

(i) Staining

(ii) Preparing a streak plate for isolated colonies.

(2) Check all the tubes for leakage/blickage.

(3) Check the levels in the acid/alkali/antifoam reservoirs and top up if necessary.

(4) Check the air out filter for wetting, and dry it out with a flame if necessary.

(5) If the medium is foaming increase the rate of antifoam addition.

(6) Agitate the antifoam reservoir to emulsify any antifoam which has separate out.

REQUIREMENTS:

Stock culture on agar slant stored in a refrigerator

Petri dishes with sterile medium

Universals with sterile medium (Broth and/or slant)

Sterile medium containing 0.1% tween 80 (For fungi)

Sterile universals containing 0.1% tween (For fungi)

Sterile pipettes containg 0.1% tween 80 (For fungi).

APPENDIX 3

ISOLATION OF BARK-DEGRADING ORGANISMS

Bacteria or fungi capable of degrading jute cutting bark may be present in the environment but in small numbers. The classic technique for their isolation is enrichment, whereby bark is added to soil or water to enrich or promote the growth of organisms capable of utilising it as a substrate for growth. Alternatively naturally rotting cuttings will contain bark-degrading organisms in higher numbers.

METHOD OF SOIL ENRICHMENT

Take a beaker or flask containing 1-2 cm depth of fresh fertile soil and add to it cuttings with adhering bark (Trim off loose fibres). Moisten with water (Do not flood otherwise conditions become anaerobic) and cover the vessel. Examine daily until visible growth is apparent on the bark and moisten if necessary.

For enrichment of anaerobes place the vessel (Uncovered) in an anaerobic jar, and for thermophiles incubate the vessel (Tied up in a plastic bag to prevent drying) at 55°C.

ISOLATION OF DEGRADING ORGANISMS

Isolate pure cultures of organisms from bark by preparing streak plates for isolated colonies on suitable (Selective) agar and inoculating agar slants from single colonies. For anaerobes use an anaerobic jar. For thermophiles incubate at 55°C.

Test the pure cultures obtained for their ability to degrade bark by weight loss measurements of sterilised bark in solid-state fermentations or other apropriate techniques.

APPENDIX 4

INDUCTION OF MUTANTS USING UV RADIATION

Prepare a slant of bacterial of fungal culture. For bacteria use overnight incubation. For fungi incubate until the culture has sporulated. Remove the growth from the slant by pipetting 3-4 Ml of sterile water (Containing 0.1% Tween 80 for Fungi) onto the slant and shaking until a dense suspension is obtained

Spread 0.1 Ml volumes of the suspension on dried (Suitable) agar plates, and irradiate from a set distance for periods up to 10 mins. Deep the suspension at 4°C. Examine the plates after appropriate incubation. The control (Non-Irradiated) plates should show confluent growth, whereas irradiated plates show decreasing number of survivors with increasing irradiation time. Choose the time giving up to 10 survivors per plate and use this treatment of produce mutants.

If the control does not show confluent growth the suspension is not concentrated enough, if there are more than Ca. 10 colonies per plate the period of irradiation should be increased. Ideally a survival rate of 1-2% is required.

APPENDIX 5

SOLID SUBTRATE FERMETATION (LAB-SCALE)

Weigh out enough material to cover the bottom of the vessel to be used to a depth of 3-5 CM (CA. 25G dry jute cuttings in a IL Flask is suitable), and sterilise by autoclaving. From a fresh culture sporulated if fungal) prepare a dense cell/spore suspension (Using tween 80 for fungi). If the culture is to be standardised perform plate counts on serial dilutions of the culture and store the suspensions at 4°C. When plate counts can be read calculate the concentration of organisms and adjust the concentration to the required value.

Using aseptic technique pipette the appropriate volume of culture as evenly as possible over the cuttings (so as to give the required inoculum level and required moisture content). Incubate the vessel in a large polythene bag containing moist tissue/cotton wool to prevent drying and agitate daily to maintain aerobic conditions.

APPENDIX 6

ESTIMATION OF MICROBIAL POPULATION IN CUTTINGS

PREVIOUS TO SAMPLING PREPARE THE FOLLOWING:

1)	Sterile 250 ml flasks	(For collecting samples)
2)	Sterile paper trays in a petri Dish	(For weighing cuttings)
3)	Sterile 250 ml flasks containing 100 ml tap water	(For washing cuttings)
4)	Sterile test tubes or flasks	(For making dilutions)
5)	Sterile tap water	"
6)	Sterile plugged pipettes (placed tip inwards in the cannister) or sterile autopipette tips (placed tip down wards in a test tube or a flask and autoclaved at 10 PSI for 15 mins)	"
7)	Sterile petri dishes	(For pour plates)
8)	Sterile medium (Stored if necessary in a refrigerator)	"

ON THE DAY OF SAMPLING:

(1) Soak forceps and scissors in alcohol

(2) Melt the sterile medium and cool in a water bath at 45°C.

SAMPLING PROCEDURE

(1) Remove a sample of cuttings from the bin and place in a sterile flask.

(2) Remove samples with forceps flamed in alcohol and weigh out 10G lots (using sterile paper trays and scissors flamed in alcohol).

(3) Place 10G cuttings into 100 ml sterile water and shake vigorously for 15 mins.

(4) Using aseptic technique prepare serial tenfold dilutions (By transfering 1 ml into 9 ml and so on).

(5) Prepare replicate pour plates of suitable dilutions by pipetting 1 ml diluted samples into an empty sterile petri dish, pouring on 15-20 ml medium at 45°C and gently mixing the contents.

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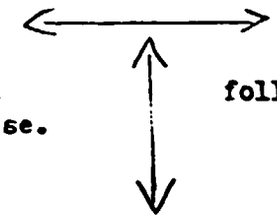
Try 5 motions in one direction



Followed by 5 motions at 90° to it

followed by 5 circular motions clockwise then anticlockwise.

(6) Allow the agar to solidify, and incubate plates for 24-72 hours until the counts are maximum.