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# Biotechnological Application of Enzymes for Making Paper Pulp from Green Jute/Kenaf

## Final Report

(1 October 2000 – 30 September 2003)

**Project code**: CF/RAS/00/153

**UNIDO Contract No.**: 2001/109

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# SYNOPSIS

It is clarified that mildewing of kenaf is caused by 32 infective fungi strains.

S4 was selected among 10 preliminarily proved suitable strains as the most suitable strains. White rot fungi treatment would help reduce chlorine requirement by about 8%. Xylanase treatment shortly after kraft pulping would also help reduce chlorine requirement by 3.7%. But the pulp yield was rather low. Maybe bio-mechanical pulping for kenaf should be studied.

Relative humidity (RH) in the storage environment and water content in kenaf are two most important factors resulting to mildewing of kenaf. The higher the RH, the easier kenaf gets mildewing. If the moisture of kenaf was controlled below 25%, RH was controlled under 57%, kanef wouldn't mildew. But RH is difficult to control in open environment, so the only way to have kenaf free from mildewing is trying to have kenaf wind dried to less water content as can.

Fungicides are effective to control of kenaf mildewing. The best is Horizon. But whether the fungicides are affecting the growth of bio-pulping micro-organisms remains to be further studied.

# Contents

# 1.INTRODUCTION

### 1.1 The project background---objectives for IBFC

In the framework of the contract with UNIDO, the objectives for IBFC are 1.4, to isolate suitable micro-organism for bio-pulping and development of enzymes from the isolated micro –organisms for bio-bleaching, 1.5, to make a comparative study of the effect of isolated micro-organic strains, 3.2, identify suitable methodology for storage of green kenaf, 2.1,application of optimal strains and enzyme extracts for preparing hand sheets, and 4.1,large scale trial application of enzymes.

### 1.2 the purpose of the report

This report is about the achievement of the above mentioned project undertaken by IBFC and constrains encountered.

# 2.THE ACHIEVEMENT OF IBFC.

We selected 2 promising strains named S4, *Trametes sanquinea* (L:Fr) Lloyd, and S6, *Tyromyces pubescens* (Schum:Fr·) Imaz which could colonize kenaf easily and suitable for bio-pulping for kenaf. We also comparatively studied the 8 strains supported by Dr. Mohiuddin, the Project Leader and found S4, *F. lignosus* work very well. The biokraft pulping with S4 was optimized. The bio-bleaching effect treated with xylanase and pollution charge were also evaluated. 32 strains causing mildew during kenaf storage were classified. The two main factors, namely RH and water content of kenaf, playing an important role in mildew of kenaf. We selected several effective fungicides to control mildew during storage Also we got ways for storage.

# 3.RESULTS AND DISCUSSION

## 3.1 Storage and mildew control of kenaf

### 3.1.1 Classification of mildew strains

**Materials**

Mildewed kenaf stem were sampled in Yuanjiang of HunanProvince and Huainan of Anhui Province. Fresh kenaf was collected in Yuanjiang.

**Mediums**

Two mediums were applied in the study.

PDA: Potato 200g, Glucose 20g, agar 20g, water 1000ml, natural PH. Autoclaved for 20min under the condition of 121℃ and 15psi. Little lactic acid was added to control bacteria growth.

Czapek: :Glucose 30g, $NaNO_3$ 2g, $KH_2PO_4$ 1g, KCl 0.5g, $MgSO_4 \cdot 7H_2O$ 0.5g, $FeSO_4$ 0.01g, agar 20g, distilled water 1000ml, natural PH. Autoclaved for 30min under the 10 psi.

**Mildew fungi separation**

Little mildew kenaf was put into a sterilized flask which contained 50ml of 0.89% physiological salt water, 0.025ml of Teween 80. Then the flask was shaken for 30min to have spores and hyphae totally separated. The PDA plates inoculated with the above liquor were incubated under 30℃ for 2~3 day to collect purified fungal strains. Czapek medium was used to get typical clone. Purified strains were kept on PDA slants and refrigerated until use.

**Inoculation and classification**

The refrigerated strains were activated before use and were inoculated on sterilized kenaf stem. If mould appeared and the fungus separated from it was identified the same as the inoculated, the strains was then classified.

After our careful systematic study, 32 primary mildew strains were separated, identified and classificated. They were in 20 genus from Zygomycotina, Ascomycotina and Deuteromycotina.The main mildew strains were *Alternaria alternata* (Fr.) Kei, *Aspergillus niger* Van Tiegh, *Botryis cinerea* Pers, *Fusaium eguiseti* (Corda) Ssce, *Rhizopus Stolonifer* Vuill, *Chaetomium funicolum* Cooks, *Chaetomium globosum* Kunze ex Fr., *Colletotrichum globosporiordes* Penz, *Curularia lunata* Boed, *Fusarium merismoides* Cda, *Fusarium solani* (Mart) Sacc, *Trichothecium roseum* (Bull) Link, *Ascochyta gossyppi* Syd. Of these, *Alternaria alternate* (Fr.) Kei, *Aspergillus niger* Van Tiegh, *Botryis cinerea* Pers, *Fusaium eguiseti* (Corda) Ssce, *Rhizopus Stolonifer* Vuill were dominant strains and *Chaetomium funicolum* Cooke, *Chuetomium globosum* Kunze ex Fr., *Colletotrichum globosporiordes* Penz, *Curularia lunata* Boed, *Fusarium merismoides* Cda, *Fusarium solani* (Mart) Sacc, *Trichothecium roseum* (Bull) Link, *Ascochyta gossyppi* Syd were accessory strains. All above samples and photos were kept by Plant Protection Research Division, IBFC.

Table 1. Main mildew strains during kenaf stem storage

| Dominant | accessory | |
|---|---|---|
| 1. *Alternaria altenata* (Fr.) Kei | 6. *Chaetomium funicolum* Coke | 11. *Fusarium solani* (Mart) sacc |
| 2. *Aspergillus niger* Van Tiegh | 7. *Chaetomium globosum Kunze ex* Fr. | 12. *Trichothecium roseum* (Bull) Lin |
| 3. *Botrytis cinerea* Pers | 8. *Colletotrichum gloeosporioides* Penz | 13. *Ascochyta gossypii* syd |
| 4. *Fusaium eguiseti* (Corda) Ssce | 9. *Curularia lunata* Boed | |
| 5. *Rhizopus stolonifer* Vuill | 10. *Fusarium merismoides* Cda | |

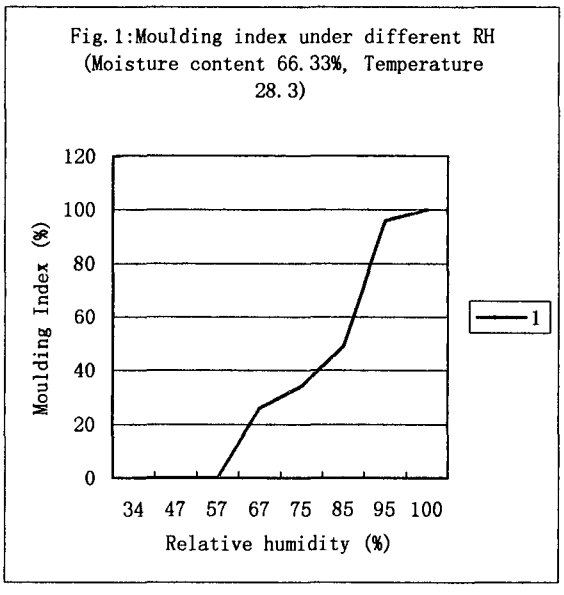
**Patten of mildewing**

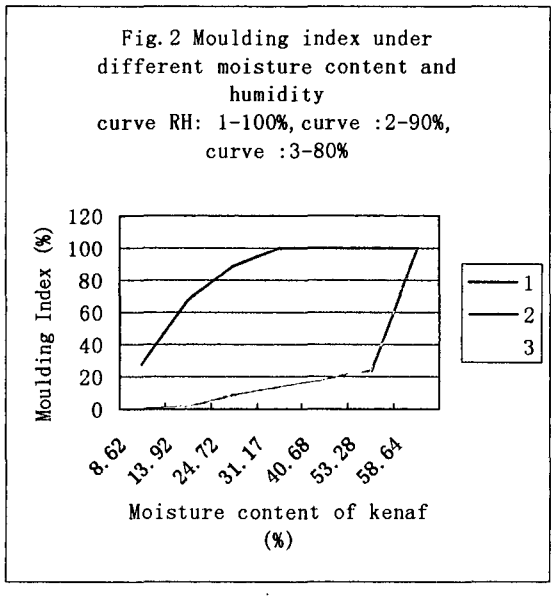
The above 32 identified strains could be classified into 3 groups according to their source. Group 1 is classified as latent infective strains including *Botrytis Cinerea* Pers, *Colletotrichum hibisci* Poll and *Helminthosporium* sp. etc. They infect kenaf while it is growing, but they don't mould. Group 2 includes *Curularia lunate* Boed, *Alternaria alternata* (Fr.) Kei which infect kenaf through wounds and natural porosity during storage period. Group 3 is saprophytic fungi including *Aspergillus* sp and *Chaetomium* sp. Mildew strains from group 1 stack along with harvesting, transporting and piling. The universally existed strains from group 2 and 3 could lead to mildew of kenaf if the environment was suitable. It demonstrated that the source of primary mildew organism was complicated and was unpredictable.

## 3.1.2 Environment for the growth of mildew strains

It is known that optimal temperature for mildew strains is 25~30℃. But there exist many kinds of thermophilia strains. So it is very difficult to control the growth of mildew organisms by regulating temperature of kenaf stack in the open air. The current study was focused on moisture.

Relative humidity (RH) of environment is the key to mildewing (Fig.1 and 2). The research with kenaf of water content of 66.33% under RH 100%, 95%, 85%, 75%, 67%, 57%, 47%, 34% demonstrated that if RH was controlled under 57%, the kenaf wouldn't mildew, if RH was increased to 67%, the kenaf would gradually mildew, and if RH was increased even more, the kenaf would mildew more severely (Fig.1). From Fig.2 we could find that the kenaf of moisture only 13.92% and 8.26% would mildew under the condition of RH 100% and the mildew index was 68% and 27% respectively. It meant that mildewing of kenaf was affected by its water content and RH during storage. The higher water content of kenaf and environment RH, the easier kenaf got mildew and faster the mildew strains grew. If the moisture of kenaf was controlled below 25%, RH was controlled under 57%, the kenaf wouldn't mildew even if abundant mildew strain resource existed.




It also demonstrated that temperature beneficial to the growth of mildew strains. Temperature could help the growth of mildew strains only under suitable RH.. In other words, in the range of 0~40℃, the higher the temperature, the faster the mildew strains grow..

And also the longer the kenaf stored, the more severe the mildew got under the same condition.

***Mildew index:***

*5 degrees are set. Degree 0 means no mildew or not obvious. Degree 1 means that mildew area accounts for less than 25% and the bark can't be separated from core. Degree 3 means that the mildew area accounts for 51—75% or the bark can't separated from core although the mildew area accounts for more than 76%. Degree 4 means that the mildew area accounts for more than 76% and more than 25% stems can have bark separated from core.*

***Mildew index*** $= \Sigma \{(\text{mildew degree} \times \text{stems})/(\text{total stems sampled}) \times 4\} \times 100.$

### 3.1.3 Selection of fungicides

Fungicides are widely used in agriculture and industries to control mildewing. Whether they are effective in control the mildewing of kenaf is what we studied. We carried out both laboratory and open air trials.

Of the 8 fungicides preliminarily selected, 4 effective fungicides were re-tested. The4fungicides were Flusilaz (flusilazole 40%), Mancozeb(ionic coordination of zinc and manganese ethylenebisdithiocarbamate+5-methyl-(4-phenoxyphenyl)-3-phenylamino-2,4-oxazolidinedione), Horizon 430sc and Thiophamate methyl. The first 2 were produced by Doupon. The third one was a product of Bayer. The last one was produced in China. The concentrations of each treatment are 1:1000 and 1:500.The kenaf was sprayed with above mentioned fungicides just after harvest. Under RH of 100%, we found that there appeared little mildew on kenaf even stored as long as 4 monthes in Horizon treatment, a little in Flusilaz and Mancozeb treatments, and Thiophamate methyl behaved differently--the mildew control ability will be gradually lost.

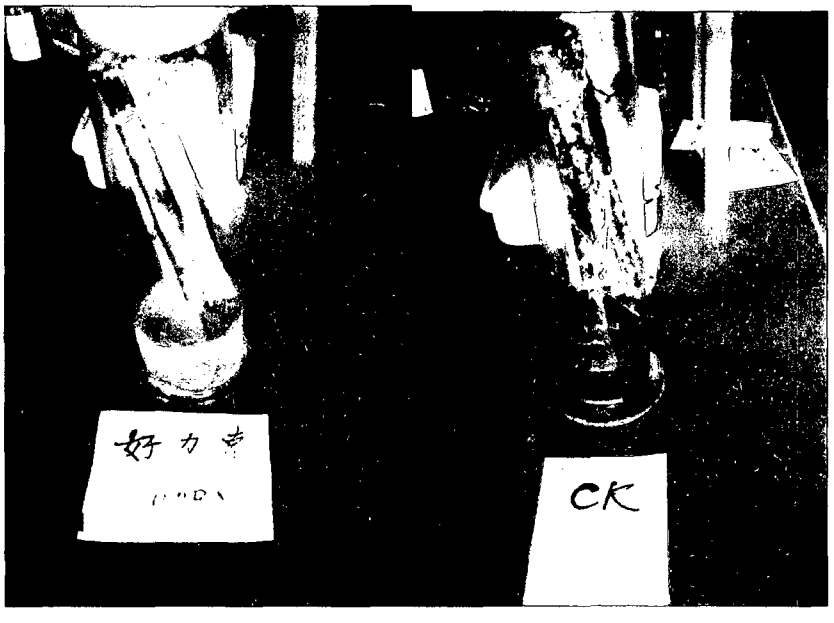



Fig. 3.Horizon Fig. 4 CK

All treatments by fungicides stored in the open air showed effective to control mildewing of kenaf. But the best was a combination of Flusilaz + Horizon. The mildew area on the kenaf was less than 10% and the color of kenaf was yellow green. The mildew area of other treatments were less than 20%. So we suggest that the combination use of Flusilaz and Horizon be used just after harvest

But whether the fungicides adversely affect the growth of white-rot fungi still remains to be studied.

### 3.1.4 Storage of kenaf

From our study we found that all treatments with fungicides would play active role in mildew-preventing of kenaf. We did some small scale storage experiments . After kenaf harvested and dried in the open air with water content below 15%, kenaf was then bundled in a diameter about 30 cm and stored. The kenaf bundles were placed on brick racks. The bundles were placed layer cross layer with holes in each layer for ventilation. The piled kenaf was house roofed with straw or woven plastic. We found that after 9 months storage through long rainfall season, all

kenaf was still fresh and little mildew appeared. But the practise of straw covering is cheap, environmental friendly and easy to use. But straw should be braided and fixed.

### 3.2 Kraft pulping conditions

Under the digesting condition of alkali requirement (NaOH) 20%, sulfidity 25%, liquor/sample 6:1, cooking temperature was ramped to 171℃ in 60min and remained for another 55 minetes. A 15 litres electric digester was used.

### 3.3 Isolation of fungi suitable for bio-pulping

Since white-rot fungi from different regions have different lignin degradation ability and the strains being studied in China are limited to *Phanerochete chrysosporium* and *Coridus versicolor,* we have to collect the wild ones from different parts of China. We collected 94 strains across China which include *Trametes suaveolens, Daedalea* sp., *Thametes sanquinea* (L:Fr.) Lloyd, *Tyromyces pubescens* (Schum:Fr.) Imaz. And *Pleurotus pulmononans*. The strains were immediately inoculated into PDA slants and incubated at 28℃ and 65%RH. After purification several times, uncontaminated strains were kept refrigerated in PDA for further study. After intensive selection both from bio-pulping ability and physiological property, we chose S4, or *Thametes sanquinea* (L:Fr.) Lloyd and S6, or S6, *Tyromyces* (Schum:Fr.) Imszn for further study. We also got 8 strains from the project leader Dr. Mohiuddin.

**Kenaf**

500 grams (OD) of cut kenaf(1.0~1.5 cm long) was packed into plastic bags (PE bag) with kraft paper of weight 60g/m$^2$ as cover. The moisture content was adjusted into 66% on wet basis and then the packed kenaf was autoclaved for 20min at 121℃ and 15psi. After cooling to room temperature, the kenaf were inoculated with fungi inoculum.

**Fungal strains**

The strains were inoculated into PDA slants and incubated at 28℃for *C. subvermispora* , 35℃ for *F. lignosus* and S4 and 38℃ for *P.chrysosporium* and 65%RH before transferred into 500ml flask containing 30ml PD. The strains were cultivated under the above mentioned temperatures

**Inoculum preparation**

1.5 grams of mycelium of each strain from flask were thrashed with a beater and mixed with sterilized water containing 50mg KH2PO4, 225mg MgSO4, 50μg FeSO4, 10μg CuSO4, 5 μg ZnSO4, 5μg MnSO4, 50μg CaCl2, 5μg VB1,10 g glucose. Then the inoculum was poured onto the packed kenaf. The treated kenaf was colonized for 2 weeks before pulping.

#### 3.3.1.Hyphae growth

The faster mycelium grows, the better the strain is. We found that *Fomes lignosus* and S4 grow very rapidly both on PDA (PD) and kenaf. Only 5 days after inoculated on to PDA, the plate and flask would be totally covered with mycelium. 4 days after inoculation with S4, the kenaf would also totally covered with mycelium. But other strains can't grow so quickly as these two strains. It take more time to have plate and flask totally covered with mycelium. Also the mycelia of these strains are different from *Fomes lignosus* and S4. The mycelia of *Fomes lignosus* and S4 are longer, wider and densely knitted while others thin, short and sparcely distributed. This means that maybe *Fomes lignosus* and S4 are the best strains for bio-pulping for kenaf.

#### 3.3.2.Sample loosening

It was confirmed that hyphae grows through lumen and parenchyma (*M.Akhtar,* 1998). We checked the treated kenaf chop over untreated kenaf by hand and found all chips treated with strains were soft and could be easily twisted into small pieces, but the untreated was still hard. This means that maybe the treated kenaf is more suitable for mechanical pulping. The energy saving and obvious strength properties enhancement should be resulted from it.

**3.3.3. Enzyme activity**

The three most important enzymes play key role are lignin peroxidase, LiP, Mn-depent peroxidase, MnP and laccase, Lac. The enzyme activity may in some degree predict and explain the bio-pulping ability of a strain. Generally speaking, these enzymes are inductive and they will response differently to different media. We had four different treatments: PG, nutritious solution with glucose + kenaf, nutritious solution without glucose + kenaf, kenaf. And we checked enzyme activity 15, 25, 30 days respectively. The enzyme activity varied greatly (Tab.1, Fig. 1). From Fig.1we found S4 and C3 exhibited comparatively strong laccase activity in PG. S4 also had weak LiP activity. *F. lignosus* only exhibited weak Laccase activity. From Table 1 we can find that each enzyme responded differently to different treatment for different strain. Some enzymes of some strains exhibited high activity after 15 days treatment, for example, Lac for STA and C2C had highest value after 15 days fermentation. But some enzymes for some strains showed highest activity after 25 days cloning, for example, the LiP for P3B, STA,S4B. Some strains. It seemed that the nutrition solution and glucose played some role in the enzyme activity. But glucose didn't work as well as nutritious solution. Only judged from enzyme activity can we preliminarily concluded that S4, C2 and ST were the strains with strong enzyme activity.

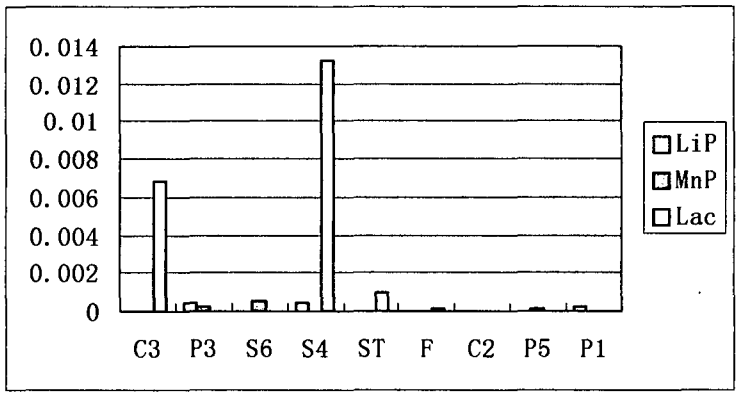



**Fig.5 Enzyme activity of different strains under PG**

C3: *C.subvermispora-3* , P3: *P.chrysoporium*, S4: *Trametes sanquinea* (L:Fr) , ST: From India, F: *F. lignosus,* P5: *P.chrysoporium*-5, C2: *C.subvermispora-2,* P1: *P.chrysoporium*,

**Table2.Enzyme activity of different strains under different treatment**

| | 15days | | | 25days | | | 30days | | |
|---|---|---|---|---|---|---|---|---|---|
| srains/Treatment | Lip | Mnp | Lac | Lip | Mnp | Lac | Lip | Mnp | Lac |
| P3A | 0 | 0.00096 | 0.002 | 0.00388 | 0.00064 | 0.002 | 0.00129 | 0.00032 | 0 |
| P3B | 0 | **0.00765** | **0.00701** | **0.01034** | **0.00287** | 0 | 0.00129 | 0.00096 | 0 |
| P3C | 0 | 0.00319 | 0.00033 | 0.00129 | 0.00064 | 0 | 0 | 0.00191 | 0 |
| STA | 0.00129 | 0.00605 | **0.01569** | **0.01939** | 0.00191 | 0.00501 | 0.00258 | 0.00032 | 0 |
| STB | 0.00258 | 0.00605 | 0.00334 | 0.00517 | 0.0035 | 0.01202 | 0.00388 | 0 | 0 |
| STC | 0 | 0.00382 | 0.00234 | 0.00388 | 0.00064 | 0.003 | 0.00258 | 0.00096 | 0.00267 |
| P1A | 0 | 0.00414 | 0.00033 | 0.00129 | 0.00159 | 0 | 0.00258 | 0 | 0 |
| P1B | 0.00129 | 0.00287 | 0 | 0.00646 | 0.00096 | 0 | 0.00258 | 0.00127 | 0 |
| P1C | 0 | 0.00127 | 0 | 0.00775 | 0.00032 | 0 | 0.00517 | 0 | 0 |
| FA | 0.00258 | 0.00287 | 0.00134 | 0.00646 | 0.00191 | 0.00334 | 0.00258 | 0.00096 | 0.00167 |
| FB | 0 | 0.00223 | 0.002 | 0.00388 | 0.00223 | 0.00334 | 0 | 0.00096 | 0.00167 |
| FC | 0 | 0.00446 | 0.00367 | 0.00517 | 0.00191 | 0.00401 | 0.00129 | 0.00127 | 0.00167 |
| S4A | 0 | 0.00319 | 0.00067 | 0.00775 | 0.0035 | 0.002 | 0.01034 | 0.00255 | 0.00067 |
| S4B | 0 | 0.00573 | 0.00067 | **0.05428** | 0.00414 | 0.00267 | 0.00129 | 0.00223 | 0.001 |
| S4C | 0 | 0.00605 | 0.00033 | **0.01163** | 0.0035 | 0.00267 | 0.00388 | 0.00159 | 0.001 |
| S6A | 0.00129 | 0.00478 | 0.00234 | 0 | 0.00159 | 0.00267 | 0.00129 | 0.00127 | 0.00067 |
| S6B | 0.00129 | 0.00223 | 0.00134 | 0 | 0.00159 | 0.00033 | 0.00258 | 0.00159 | 0.00167 |
| S6C | 0 | 0.00319 | 0.00167 | 0.00129 | 0.00191 | 0.00134 | 0.00517 | 0.00223 | 0.00167 |
| C2C | 0 | **0.01211** | **0.01669** | 0 | 0.00319 | 0.00534 | 0.00258 | 0.00159 | 0.00134 |

A: nutritious solution with glucose, B: nutritious without glucose, C: no nutritious and glucose

### 3.3.4. pulp yield

We toke 5 strains that well colonize kenaf for pulping test. But we found that all pulp yields were rather low, esp. screened pulp yield, compared to kenaf untreated with fungi. From Fig.2 we found the unscreened and screened pulp yield for *F .lignosus* which showed the highest pulp yield were 46.7 and 36.8% respectively, 5.5 and 10.1 percentage lower than the CK. S4 also had a higher pulp yield. This is conform to M.Akhtar"s observation. Judging from pulp yield, we drew a conclusion that these two strains may be the most suitable strains for kenaf cloning.

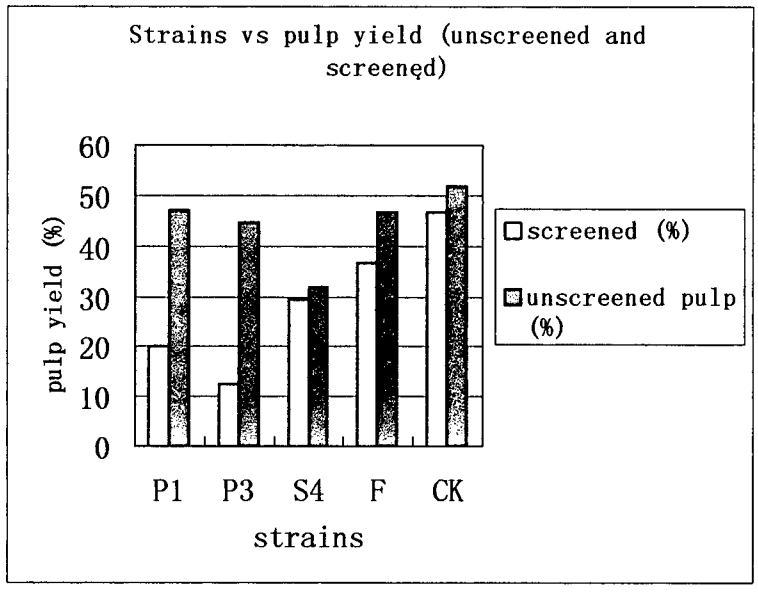



Fig.6 Pulp yield of kenaf treated with different strains

### 3.3.5 Physical properties

From Fig.3 we could find that all physical properties of pulps from fungi treated kenaf were worse than untreated. From Fig.4 we found the Kappa values for most strains were higher than CK except for S4. That for *F.lignosus* was a little than CK. This is the same as M. Akhtar"s report.

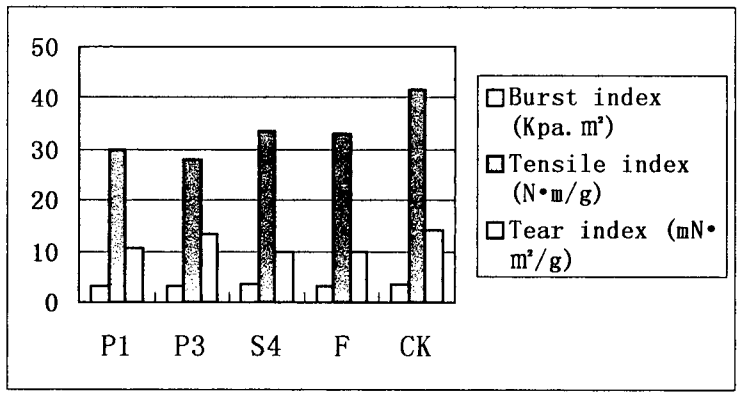



Fig.7 Physical properties of different strains

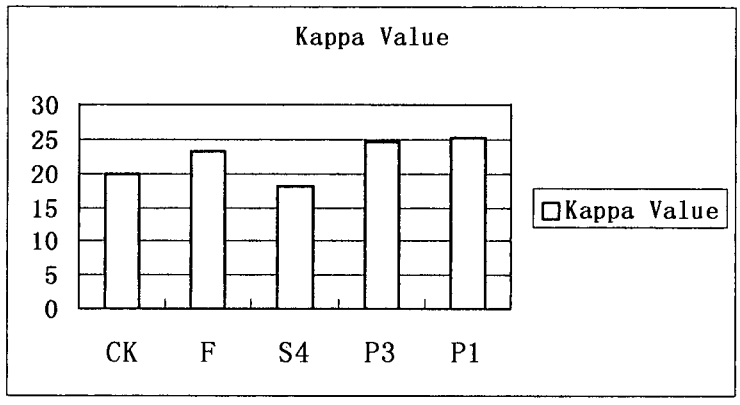



Fig.8 Kappa value of different strains

The brightness (Fig.5) of pulps from different strains also varied greatly. P1 and P3 exhibited higher brightness over CK but S4 and *F .lignosus* had lower brightness (Fig.6). Opacity and freeness varied little (Fig.7).

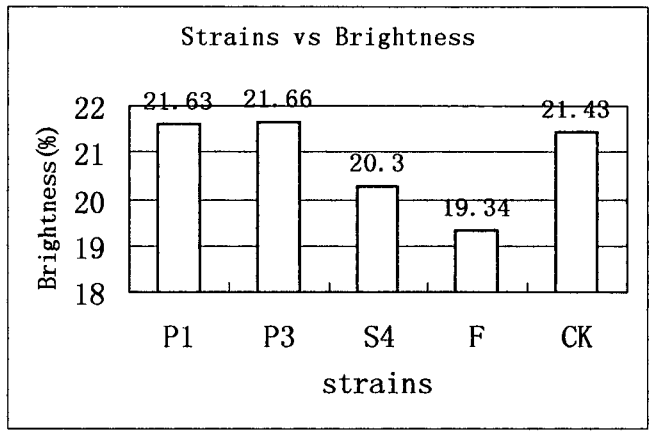



Fig.8 Brightness of unbleached pulp of different strains

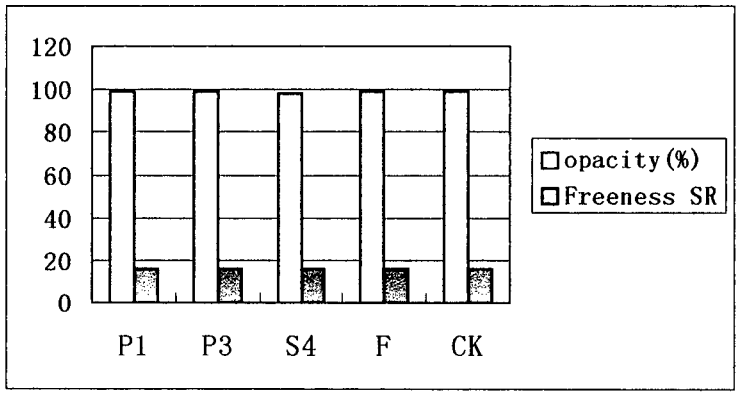



Fig.10 Physical properties of different strains

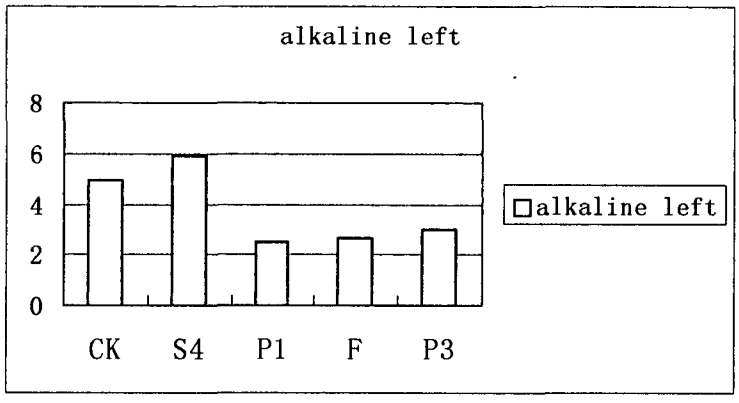



Fig.11 Alkaline left of different strains

The alkaline left o different strains changed greatly. That for S4 was almost 6 g/l. This means that less alkaline will be needed during kraft pulping.

### 3.3.6 Bleaching

From Fig.8 we find that the brightness for bleached pulp acted not the same. Other strains except S4 had negative role in brightness. But more work was needed in multi-stages bleaching. The chlorine left for S4 was also higher (Fig.9).

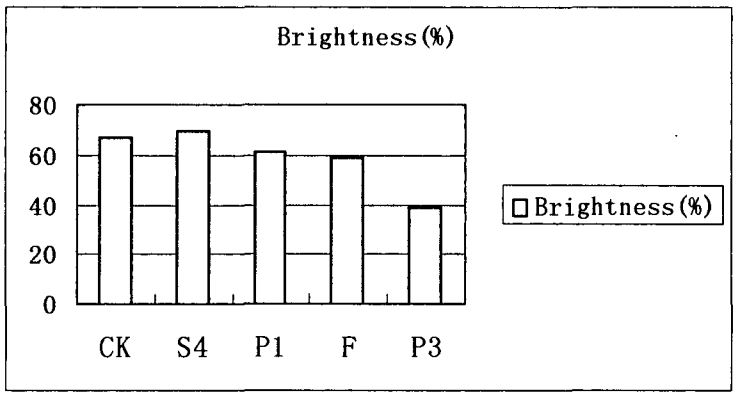



Fig.12 Brightness of Chlorine bleached pulp of strains

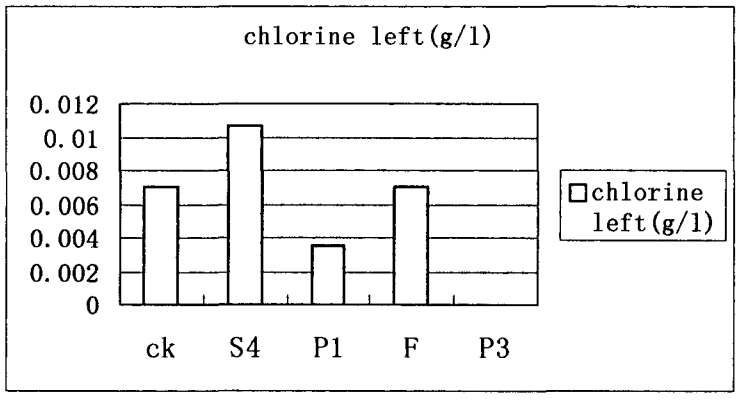



Fig.13 Chlorine left of strains.

From the above analysis S4 and *F .lignosus* can be selected as most suitable strains for kenaf. But the pulp yield and some physical properties are not so good. More work is needed.

### 3.4 Bio-bleaching of kraft pulps

According to the research requirement, we did some bio-bleaching studies both for kraft and bio-kraft pulps.

The xylanase is a product of Hunan New Century Biochemical Co.Ltd, a Sino-USA company, with optimum temperature 50℃ and pH value 6.5. We found the optimum dosage is 5 IU/g (Table 3).

Table 3. Enzyme dosage optimization

| Conditions | Enzyme dosage IU/g pulp | | | | |
|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 |
| Pulp consistency % | 5 | 5 | 5 | 5 | 5 |
| Temperature℃ | 50 | 50 | 50 | 50 | 50 |
| Time, Minutes | 60 | 60 | 60 | 60 | 60 |
| Initial kappa no. | 21.54 | 21.54 | 21.54 | 21.54 | 21.54 |
| Final kappa no. | 20.45 | 20.38 | 20.18 | 20.03 | 20.01 |

From Table 4 we found that xylanase helpt the process of bleaching, the kappa number after enzyme treatment would be reduced from the original 21.33 to 18.93,or a reduction of 2.4 for fungi treated kenaf and 0.84 for not fungi treated. This means that white rot fungi would help the modification or dissolving of lignin in the coming process and help reduce the chlorine requirement. Compared to check, the chlorine requirement for white rot fungi kraft kenaf pulp would reduce by about 8% and the final whiteness would increase by about almost 3%. But it's strange that the COD didn't show any improvement after white rot fungi treatment.

Considering the pulp yield, esp. the fine pulp yield for white rot fungi treatment is rather low, around 30% and no improvement in physical properties. We think there is much work to do before we are going to do large scale trial production. And also, we tentatively suggest that much work should be done on bio-mechanical pulping of kenaf. Dr. M. Akhtar from Forest Products Laboratory(FPL), USDA once reported the similar result when doing bio-kraft pulping of kenaf and he also reported inspiring result on bio-mechanical pulping. From our observation, we found that the kenaf core would soften and easily be grounded, and the kenaf bast fiber would be well separated in 14 days after treated with S4.

Table 4 results of bleaching experiments in DED sequence with enzyme of kraft pulp

| Stages | conditions | Check | Not fungi treated | Fungi treated |
|---|---|---|---|---|
| x | Pulp consistency | 5 | 5 | 5 |
| | Temperature℃ | 50 | 50 | 50 |
| | Initial pH | 6.5 | 6.5 | 6.5 |
| | Time, minutes | 60 | 60 | 60 |
| | Initial kappa no. | 20.54 | 20.54 | 21.33 |
| | Final kappa no. | 20.49 | 19.70 | 18.93 |
| D | $ClO_2$(as $Cl_2$)% | 2.70 | 2.60 | 2.50 |
| | Pulp consistency % | 7 | 7 | 7 |
| | Temperature ℃ | 70 | 70 | 70 |
| | Time, minutes | 60 | 60 | 60 |
| | Residual $Cl_2$ in effluent % | 0.43 | 0.42 | 0.40 |
| | Brightness % ISO | 40.19 | 40.23 | 41.15 |
| E | NaOH % | 2 | 2 | 2 |
| | Pulp consistency % | 5 | 5 | 5 |
| | Temperature ℃ | 60 | 60 | 60 |
| | Time, minutes | 60 | 60 | 60 |
| D | $ClO_2$(as $Cl_2$)% | 1.80 | 1.73 | 1.66 |
| | Pulp consistency % | 7 | 7 | 7 |
| | Temperature ℃ | 70 | 70 | 70 |
| | Time, minutes | 60 | 60 | 60 |
| | Residual $Cl_2$ in effluent % | 0.42 | 0.42 | 0.43 |
| | Bleaching yield % | 95.45 | 95.55 | 94.75 |
| | Brightness % ISO | 78.31 | 80.13 | 81.25 |

## 4.conclusions

1. Mildewing of kenaf is caused by 32 infective fungi strains.
2. S4 was selected among 10 preliminarily proved suitable strains as the most suitable strains. White rot fungi treatment would help reduce chlorine requirement by about 8%. Xylanase treatment shortly after kraft pulping would also help reduce chlorine requirement by 3.7%. But the pulp yield was rather low. Maybe bio-mechanical pulping for kenaf should be studied.
3. Relative humidity (RH) in the storage environment and water content in kenaf are two most important factors resulting to mildewing of kenaf. The higher the RH, the easier kenaf gets mildewing. If the moisture of kenaf was controlled below 25%, RH was controlled under 57%, kanef wouldn't mildew. But RH is difficult to control in open environment, so the only way to have kenaf free from mildewing is trying to have kenaf wind dried to less water content as can.
4. Fungicides are effective to control of kenaf mildewing. The best is Horizon. But

whether the fungicides are affecting the growth of bio-pulping micro-organisms remains to be further studied.

References

Masood Akhtar, Environmentlly Friendly Technologies for the Pulp and Paper Industry, John Wiley&Sons, Inc.1998

H·S· Sabharwal, etc·, BioMechanical Pulping of Kenaf, Tappi·J· 77(12): pp105~112.

H.S. Sabharwal,etc·, Refiner Mechanical and Biomechanical Pulping of Jute, Holzforschung, 49(6), pp:537~544

Xobayashi.Y. and Matsuo.R., Celluose chem. Technol. 15:639

Yu.H., ect. Pulp and Paper Manufacture:Application and Fundamental Investigations, Butterworth Heinemann, 1990,P59.

Yunxiong Zhang, The Development Direction for Bio-pulping of Kenaf, !st Workshop for Making Paper Pulp from Kenaf, Yuanjiang, Hunan,China,1999.

Wang Chaoyun, ect. , Present status and Prospect of Biotechnological Application for Making Pulp from Kenaf, Proceedings of the First International Workshop on Paper Production from Jute and Kenaf, IJO, pp11~15

# Executive Summary of All Activities

## IBFC

| Date | Activity | Result |
|---|---|---|
| Dec.2000 | Inauguration and Programming meeting held in Dhaka, Bangladesh | |
| 1st Qtr,2001 | Made preparations for the undertaking of the project. | Chemicals, reagents, equipments bought or installed. |
| 2nd Qtr,2001 | Activity 1.4(a) Strains collection across China. | 90 strains collected across China and preliminary study made to judge their bio-pulping abilities on kenaf. |
| 2nd Qtr 2001 | Activity1.4(b)Collection of strains for wood | 2 strains collected in China. |
| 3rd,Qtr 2001 | Activity 1.4(c). Preliminary selection of suitable strains for kenaf. | 2 strains named S4,*Trametes sanquinea,* and S6, *Tyromyces pubescences* were screened. |
| 3rd and 4th Qtr,2001 | ①Activity 3.2(l) Collection of green jute. | 5 tons of green jute were collected and stored. |
| | ②Activity 3.2(m) Selection of fungicides | Horizon. Proved to be the best. |
| | ③Activity 3.2(n)Storing method | Method proposed. |
| | ④Mildewing strains classification | 32 mildewing strains were clarified and classified. |
| | ⑤ Environmental factors affecting mildewing clarified. | RH of air and water content of kenaf were the main factors affecting mildewing. |
| | ⑥Midterm progress evaluation forum | Attended the forum held in Grenoble,France. |
| | ⑦ Strains collection from Dr. Mohiuddin | 8 Strains sent to IBFC by Dr.Mohiudin in December. |
| 1st Qtr 2002 | ① Activity 1.1(c).Selection of the most suitable strain from the strains screened by IBFC and donated by Dr.Mohiuddin. | S4 and *F. lignosus* confirmed the most suitable ones for kenaf for bio-kraft pulping. |
| | ②Activity 1.5(e). Growth parameters study. | Determined the optimal growth parameters for S4. |
| 2nd Qtr and 3rd Qtr 2002 | ① Activity 1.5(d). Enzyme activity evaluation | Confirmed that for LiP, MnP and Lac of S4 were promising. |
| | ② Activity 2.1(i). Determination of pulping and bleaching yields, chemical consumption, physical and chemical properties for unbleached and bleached pulp and different physical properties of handsheets and process parameters without fungi treatment. | Optimized the pulping condition and clarified the pulp yield, physical and chemical properties. The alkali requirement (NaOH) 20%, sulfidity 25%, liquor to kenaf ratio 6:1, cooking temperature ramped to 171℃ in 60 min and maintained for 55 min. |
| | ③ Activity 2.1(i). Determination of | Clarified the physical properties of pulps with and |

| | | |
|---|---|---|
| | pulping and bleaching yields, chemical consumption, physical and chemical properties for unbleached and bleached pulp and different physical properties of handsheets and process parameters with fungi treatment. | without bleaching. |
| | ④ Activity 2.1(j). Effluent analysis | Analyzed. Chemicals including NaOH and chloride left in black liquor were higher than check. |
| | ⑤ Activity 2.1(k). Evaluation and results (yield, physical and optical properties of pulp produced) | Physical properties not shown improvement by bio-kraft pulping. But the pulp yield from bio-kraft pulping was less the check |
| | ⑥ Activity 3. 2. Further did storage experiment combining fungicides and storing method. | It was proved effective to have green kenaf safely stored by Horizon treatment and covered by straw mattress or plastic film house-roof shaped |
| 4th Qtr 2002 | Activity 5.1(p). Prepared the interim report and went to Dhaka, Bangladesh to attend the mid-term evaluation meeting. | |
| 2nd Qtr 2003 | Activity 2.1. and 4.1. Bio-bleaching trial | Bio-bleaching conditions optimized and the effect clarified. |
| 3rd Qtr 2003 | Draft final report preparation | Completed and submiteed. |

# Detailed Experimental Procedure

## IBFC

**Materials**

Mildewed kenaf stem were sampled in Yuanjiang of Hunan Province and Huainan of Anhui Province. Fresh kenaf was collected in Yuanjiang.

**Mediums**

Two mediums were applied in the study.

PDA: Potato 200g, Glucose 20g, agar 20g, water 1000ml, natural PH. Autoclaved for 20min under the condition of 121℃ and 15psi. Little lactic acid was added to control bacteria growth.

Czapek: :Glucose 30g, $NaNO_3$ 2g, $KH_2PO_4$ 1g, KCl 0.5g, $MgSO_4 \cdot 7H_2O$ 0.5g, $FeSO_4$ 0.01g, agar 20g, distilled water 1000ml, natural PH. Autoclaved for 30min under the 10 psi.

**Mildew fungi separation**

Little mildew kenaf was put into a sterilized flask which contained 50ml of 0.89% physiological salt water, 0.025ml of Teween 80. Then the flask was shaken for 30min to have spores and hyphae totally separated. The PDA plates inoculated with the above liquor were incubated under 30℃ for 2~3 day to collect purified fungal strains. Czapek medium was used to get typical clone. Purified strains were kept on PDA slants and refrigerated until use.

**Inoculation and classification**

The refrigerated strains were activated before use and were inoculated on sterilized kenaf stem. If mould appeared and the fungus separated from it was identified the same as the inoculated, the strains was then classified.

***Mildew index:***

*5 degrees are set. Degree 0 means no mildew or not obvious. Degree 1 means that mildew area accounts for less than 25% and the bark can't be separated from core. Degree 3 means that the mildew area accounts for 51—75% or the bark can't separated from core although the mildew area accounts for more than 76%. Degree 4 means that the mildew area accounts for more than 76% and more than 25% stems can have bark separated from core.*

$$\textit{Mildew index} = \Sigma \{(\textit{mildew degree} \times \textit{stems})/(\textit{total stems sampled}) \times 4\} \times 100.$$

**3.1.3 Selection of fungicides**

Of the 8 fungicides preliminarily selected, 4 effective fungicides were re-tested. The4fungicides were Flusilaz (flusilazole 40%), Mancozeb (ionic coordination of zinc and manganese ethylenebisdithiocarbamate+5-methyl-(4-phenoxyphenyl)-3-phenylamino-2,4-oxazolidinedione), Horizon 430sc and Thiophamate methyl. The first 2 were produced by Doupon. The third one was a product of Bayer. The last one was produced in China. The concentrations of each treatment are 1:1000 and 1:500.The kenaf was sprayed with above mentioned fungicides just after harvest.

**Storage of kenaf**

After kenaf harvested and dried in the open air with water content below 15%, kenaf was then bundled in a diameter about 30 cm and stored. The kenaf bundles were placed on brick racks. The bundles were placed layer cross layer with holes in each layer for ventilation. The piled kenaf was house roofed with straw or woven plastic.

**Pulping conditions**

Under the digesting condition of alkali requirement (NaOH) 20%, sulfidity 25%, liquor/sample 6:1, cooking temperature was ramped to 171℃ in 60min and remained for another 55 minetes. A 15 litres electric digester was used.

**Physical properties evaluation:**

As Tappi standard.

**Isolation of fungi suitable for bio-pulping**

**Kenaf**

500 grams (OD) of cut kenaf(1.0~1.5 cm long) was packed into plastic bags (PE bag) with kraft paper of weight 60g/m$^2$ as cover. The moisture content was adjusted into 66% on wet basis and then the packed kenaf was autoclaved for 20min at 121℃ and 15psi. After cooling to room temperature, the kenaf were inoculated with fungi inoculum.

**Fungal strains**

The strains were inoculated into PDA slants and incubated at 28℃ for *C. subvermispora* , 35℃ for *F. lignosus* and S4 and 38℃ for *P.chrysosporium* and 65%RH before transferred into 500ml flask containing 30ml PD. The strains were cultivated under the above mentioned temperatures

**Inoculum preparation**

1.5 grams of mycelium of each strain from flask were thrashed with a beater and mixed with sterilized water containing 50mg KH2PO4, 225mg MgSO4, 50μg FeSO4, 10μg CuSO4, 5 μg ZnSO4, 5μg MnSO4, 50μg CaCl2, 5μg VB1,10 g glucose. Then the inoculum was poured onto the packed kenaf. The treated kenaf was colonized for 2 weeks before pulping

**Enzyme activity**

Four different treatments: PG, nutritious solution with glucose + kenaf, nutritious solution without glucose + kenaf, kenaf. And we checked enzyme activity 15, 25, 30 days respectively.

**Method**

As the methods Dr. Mohiuddin recommended.

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