UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF KENAF (Hibiscus cannabinus) BIORETTING PROCESS BY LOCALLY ISOLATED MICROORGANISMS

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IPTPH 2014 4

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

JULY 2014

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in the fulfillment of the requirement for the degree of Master of Science

ASSESSMENT OF KENAF (*Hibiscus cannabinus*) BIORETTING PROCESS BY LOCALLY ISOLATED MICROORGANISMS

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July 2014

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Faculty: Institute of tropical Forests and Forestry Products (INTROP)

Kenaf (*Hibiscus cannabinus* L.) is a fast growing warm seasonal plant in the family of Malvaceae cultivated mainly for its fibre. The two distinct layers in kenaf stalk include the inner core and outer bast layer. The process of removing non cellulosic substances in kenaf to obtain high quality bast fibres is retting. Common method of retting is by placing kenaf in water where microorganisms degrade the pectin rich middle lamella connecting adjacent fibre cells and release bast fibre. However, the long retting time is not economically worthy and causes pollution. The aim of the study is to assess and characterize kenaf bast fibre retted by microorganisms. Following that, microorganisms were isolated from various sources of kenaf and screened for enzyme activities. The retting capacities of these microorganisms were evaluated by treating the kenaf in the monoculture of each isolates. Total of five fungi and three bacteria isolated. These isolates were identified based on morphological structures, biochemical tests and API Kit analysis. All fungal isolates were identified to be *Aspergillus* sp. The bacterial isolates were identified as *Bacillus sp* and *Sphingomonas* sp. The isolates were screened for cellulase, hemicellulase and pectinase production. The enzyme activities were determined by growing the fungal isolates in solid state fermentation and bacterial isolates in submerged fermentation. Both fungal and bacterial isolates showed higher pectinase activities compared to cellulase and hemicellulase activities. Pectinase activity of fungal isolates varies between different isolates and treatment days and the activities for all the isolates increased from third day onwards. Three isolates *Aspergillus* sp. P1, *Aspergillus* sp. P2 and *Aspergillus* sp. M1 showed higher pectinase activity (0.03 – 0.06 µg/ml/min) compared to the other two isolates, *Aspergillus* sp. P3 and *Aspergillus* sp. M2. Highest pectinase activity of bacterial isolates recorded at 12^{th} h which ranged between 0.04 – 0.09 µg/ml/min. Bioretting process was performed by treating kenaf stalk with monoculture of the fungal isolates for five days. The samples were then evaluated for fibre brightness and tensile strength. All the treated fibres showed brightness within the range of 65 – 75% based on CIE L.a.b system. The tensile strength recorded lies within the range of 150 – 500 MPa which varies between isolates on different treatment days. In conclusion, retting reached completion on third day of treatment producing fibres of considerable tensile strength and

brightness. The fibres were also easily separated and combed upon retting. Fungal and bacterial inoculums substantially improved the kenaf retting process.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Pengajian Master Sains

PENILAIAN PROSES PENGERETAN KENAF (*Hibiscus cannabinus*) OLEH MIKROORGANISMA YANG DIASINGKAN

Oleh

KOHGILAA A/P MUTHU KUMAR

Julai 2014

Pengerusi: Wan Zuhainis binti Saad, PhD
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Kenaf (*Hibiscus cannabinus* L.) adalah tumbuhan yang cepat tumbuh semasa musim panas. Kenaf berasal dari keluarga Malvaceae dan ditanam terutamanya untuk serat. Batang tumbuhan ini terdiri daripada dua lapisan yang berbeza iaitu teras dalaman dan serat bahagian luar. Proses pengeretan melibatkan pengasingan bahan-bahan bukan selulosa daripada kenaf untuk mendapatkan serat gentian yang berkualiti tinggi. Kaedah pengeretan yang biasa adalah dengan menempatkan kenaf di dalam air di mana mikroorganisma akan melepaskan sel – sel bersebelahan yang dicantum oleh kandungan pektin yang tinggi. Kaedah ini mengurangkan kandungan pektin dalam lamela tengah dan melepaskan serat bahagian luar kenaf. Walau bagaimanapun, kaedah pengeretan ini mengambil masa yang panjang dan tidak sesuai dari segi ekonomi dan menyebabkan pencemaran. Dalam kajian ini, mikroorganisma telah diasingkan daripada pelbagai sumber kenaf dan disaring untuk aktiviti enzim. Kapasiti pengeretan oleh semua mikroorganisma dinilai dengan menjalankan pengeretan kenaf menggunakan monokultur mikrorganisma yang diasingkan. Sebanyak lima spesies kulat dan tiga spesis bakteria berjaya diasingkan. Mikroorganisma yang diasingkan telah dikenal pasti berdasarkan struktur morfologi, ujian biokimia dan analisis Kit API. Kulat adalah *Aspergillus* sp. dan bakteria adalah *Bacillus* sp. dan *Sphingomonas* sp. Mikroorganisma juga telah disaring untuk kandungan enzim selulosa, hemiselulosa dan pektin. Aktiviti-aktiviti enzim kulat ditentukan melalui kaedah fermentasi keadaan pepejal dan bakteria melalui fermentasi keadaan cecair. Kedua-dua kulat dan bakteria menunjukkan aktiviti enzim pektin lebih tinggi berbanding dengan enzim selulosa dan hemiselulosa. Aktiviti enzim pektin oleh kulat berbeza mengikut spesis kulat dan masa pengeretan. Kesemua kulat menunjukkan aktiviti enzim pektin tinggi pada hari ketiga pengeretan dan hari – hari yang seterusnya. Tiga spesis kulat iaitu *Aspergillus* sp. P1, *Aspergillus* sp. P2 dan *Aspergillus* sp. M1, menunjukkan aktiviti pektin yang tinggi (0.03 – 0.06 μg/ml/min) berbanding dengan spesis kulat *Aspergillus* sp. P3 dan *Aspergillus* sp. M2. Aktiviti enzim pektin yang tinggi untuk bakteria dicatatkan pada jam ke-12 iaitu pada julat 0.04 – 0.09 μg/ml/min. Proses pengeretan secara biologi dilakukan dengan menempatkan tangkai kenaf dalam monokultur mikroorganisma selama lima hari. Sampel kemudiannya dinilai untuk kecerahan gentian dan kekuatan tegangan. Semua gentian daripada monokultur mikroorganisma menunjukkan

kecerahan antara 65 – 75% berdasarkan sistem Lab CIE. Nilai kekuatan tegangan direkodkan adalah antara 150 – 500 MPa. Nilai kekuatan tegangan berbeza bergantung kepada spesis mikroorganisma dan masa pengeretan. Berdasarkan kekuatan tegangan, kecerahan serat dan analisis SEM boleh dibuat kesimpulan bahawa pengeretan yang sempurna adalah pada hari ketiga pengeretan di mana serat kenaf yang dihasilkan mempunyai tegangan yang tinggi dan cerah. Serat ini juga mudah dipisahkan dan disikat selepas pengeretan. Inokulum kulat dan bakteria meningkatkan taraf serat kenaf yang dihasilkan.

ACKNOWLEDGEMENTS

First and foremost, my heartfelt gratitude to my supervisor, Dr. Wan Zuhainis binti Saad for her guidance throughout this study. All her advice, suggestions and support was an enormous help in conducting the research and writing this thesis. My deepest appreciation also goes to my co-supervisor Prof. Madya Dr. Rosfarizan binti Mohamad for her continuous guidance for completing my study. A special thanks to my co-supervisor Professor Dr. Paridah binti Md Tahir, director of Institute of Tropical Forest and Forestry products (INTROP) for providing me the funds to conduct my research. Also, sincere thanks to all my laboratory mates and staffs from INTROP, Microbiology laboratory and Bioprocess laboratory that have been helpful towards completing my laboratory work especially Ms Nadia Abdullah and Ms Puvaneswary. My appreciation is also to En. Baharom from INTROP for guiding me in collecting kenaf samples from Taman Pertanian, UPM. Lastly, huge thanks to my husband, Mr Prasad, for his continuous encouragement, my sisters, Ms Suganthi and Ms Deepaa, for their assistance and all my family members and friends for their support.

APPROVAL SHEET

I certify that a Thesis Examination Committee has met on date of viva voce to conduct the final examination of Kohgilaa A/P Muthu Kumar on her thesis entitled "Assessment of Kenaf (*Hibiscus cannabinus*) Bioretting Process by Locally Isolated Microorganisms" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Masters of Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ASTM	American Society for testing and materials
API	Analytical Profile Index
CIE	Commission Internationale de l'Eclairage
CMC	Carboxymethyl cellulose
CMCase	Carboxymethyl cellulase
DNS	Dinitrosalicylic Acid
DP	Degree of polymerization
Endo-PG	Endo-polygalacturonase
FAO	Food and Agriculture Organization
FPase	Filter paperase
FTIR	Fourier Transform Infrared
H.	*Hibiscus*
H_2O_2	Hydrogen peroxide
h	hour
ID	Identity description
IBS	Institute of Bioscience
IMViC	Indole production, Methyl red, Voges-Proskauer and Citrate
INTROP	Institute of Tropical Forests and Forestry Products
L*a*b	Luminance , red to green, blue to yellow
LKTN	Lembaga Kenaf dan Tembakau Negara
MARDI	Malaysia Agriculture Research and Development Institute
MR-VP	Methyl Red, Voges-Proskauer
NA	Nutrient Agar

LIST OF ABBREVIATIONS

NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NB	Nutrient Broth
OD	Optical density
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PE	Pectinase esterase
PG	Polygalacturonase
PGA	Polygalacturonic acid
PMG	Polymethyl galacturonase
PNPG	*p*-nitrophenyl-β-D-glucopyranoside
PNPX	*p*-nitrophenyl-β-D-xylopyranoside
psi	pounds per square inch (pressure)
rpm	rotation per minute
SEM	Scanning Electron Microscope
SIM	Sulfide-Indole Motility
SMF	Submerged fermentation
sp.	Species
SSF	Solid state fermentation
Tappi	Technical Association of the Pulp and Paper Industry
UPM	Universiti Putra Malaysia
USSR	Union of Soviet Socialist Republic

CHAPTER 1

INTRODUCTION

Non wood fibres can be grouped into two categories; Monocots such as cereal straws, sugarcane bagasses, bamboo and corn stalk (similar to hardwood owing to the fibre fractionation similarity, but much more heterogenous and contain a large proportion of very thin walled cells, vessels and fine epidermal cells in a wide range of dimension) and dicot such as kenaf, flax, hemp and straw (contains two distinct fibre types: an inner core of short fibre surrounded by a layer of longer bast fibre) (Hurter, 1997). The chemical composition of non-wood plant fibre vary widely depending on plant, the soil and growing conditions (H'ng et al., 2009; Jahan et al., 2008; Ververis et al., 2004). In comparison with wood species, non-wood fibres have lower lignin and higher hemicelluloses and ash contents (Ashori et al., 2006).

Kenaf (*Hibiscus cannabinus* L.) is a fast growing warm seasonal plant in the family of Malvaceae. It yields a soft fibre from the stem that is very similar to jute but has certain advantages over jute. It has better adaptations to various growth condition and produce fibre crop in shorter time compared to jute. Kenaf plantation reaches its peak after World War II where kenaf used to supply cordage material for the war effort (Wilson et al., 1965). Ever since, many researches carried on kenaf to enhance fibre yield.

The bark of kenaf, which contains bast fibres (phloem tissues) and core (xylem tissues), are separated by meristematic tissue, the vascular cambium. The presence of vascular cambium interface between kenaf bast and core results in easy separation between these two components as long as the plants are recently harvested (Webber et al., 2002). Whole stalk kenaf can be used in corrugated medium (Kugler, 1988), in building materials such as particle boards and for reinforcement in injection molded and extruded plastics (Webber and Bledsoe, 1993).

Retting is a process used to remove non-cellulosic substances to obtain textile quality bast fibres. During this process, the pectin rich middle lamella connecting adjacent fibre cells degraded to release the bast fibres (Yu and Yu, 2007). Small scale farmers usually spread the harvested kenaf stem in flowing river with one end tied to the bank. After 10 to 15 days, the stem collected, washed and bast fibre stripped to be used in industries.

Preliminary retting experiments revealed that a natural bacterial population already present on the bark. Kenaf stalks retted in open plastic troughs with temperature maintained at 32±2°C throughout the process (Ramaswamy et al., 1994). Water retting, which depended on fermentation of matrix polysaccharides by anaerobic bacteria, was the primary method used formerly. Pollution from this method as well as high cost of labour and drying caused water retting to be replaced with dew retting.

The common retting method used is water retting. Harvested kenaf bast decorticated manually and placed in flowing river water or lake and left to ret for more than ten days. The long retting period is economically not worthy and at the same time causes

pollution. Following this, dew retting suggested to replace water retting. However, this method is restricted to geographical area and climate (Akin et al., 1997)

Research were done to test the efficiency of chemicals on retting process. Throughout the research, various concentration of sodium hydroxide tested for kenaf bast retting process. This method greatly improves the retting by reducing the retting period to less the three days. But somehow, it affects the strength of fibres produced and not advisable for industries requiring strong fibre (Kawahara et. al., 2004)

Enzyme retting suggested next where enzymes applied directly to ret the kenaf bast (Akin et. al., 2000). It has shown that enzyme retting produced good quality fibre in just 24 h. However, due to high cost, this method is not applicable for now. An alternative method required to produce higher grade fibre with shorter retting period. To achieve this goal, it is essential amongst other things, to set up suitable method for extracting the fibre from the stems. At present, the most promising process would appear to be to be microbiology retting (Candillo et al., 2009).

The aim of this study is to assess and characterize kenaf bast fibre retted by microorganisms. Microbial retting performed on kenaf, cultivar V36 and evaluated for strength and brightness. The following specific objectives were executed to achieve this crucial goal:

1. to isolate fungi and bacteria from various sources of kenaf and identify.
2. to screen the isolates for retting enzymes; carboxymethyl cellulase, filter paperase, β-glucosidase, xylanase, β-xylosidase and pectinase.
3. to determine the characteristics of the retted kenaf based on fibre brightness, tensile strength and SEM analysis

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