PRODUCTION AND OPTIMIZATION OF CARBOXYMETHYL CELLULASE AND FILTER PAPERASE OF LOCALLY ISOLATED Streptomyces lucitanus C5/2 UNDER SUBMERGED FERMENTATION OF NAPIER GRASS

SHAHIRAH BINTI MANAP

IPTPH 2017 1

PRODUCTION AND OPTIMIZATION OF CARBOXYMETHYL CELLULASE AND FILTER PAPERASE OF LOCALLY ISOLATED *Streptomyces lucitanus* C5/2 UNDER SUBMERGED FERMENTATION OF NAPIER GRASS

By

SHAHIRAH BINTI MANAP

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

January 2017

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

PRODUCTION AND OPTIMIZATION OF CARBOXYMETHYL CELULLASE AND FILTER PAPERASE BY LOCALLY ISOLATED *Streptomyces lucitanus* C5/2 UNDER SUBMERGED FERMENTATION OF NAPIER GRASS

By

SHAHIRAH BINTI MANAP

January 2017

Chairman : Assoc. Prof. Rosfarizan Mohamad, PhD
Institute : Tropical Forestry and Forest Product

Lignocellulosic biomass materials are known as a sustainable, renewable feedstock for biofuels production due to domestically abundant and low-production-cost derived from non-food sources. However, due to complex molecular structures of the cellulose, it becomes difficult to hydrolyze into fermentable sugar as the feedstock. The bioconversion of cellulose to fermentable sugar can be catalyzed by a group of enzymes known as cellulolytic enzymes, which is commonly produced by microorganisms (fungi, bacteria, and protozoa). Cellulolytic enzymes system mainly comprised of endoglucanase (carboxymethyl cellulase), exoglucanase, and β-glucosidase enzymes which react together to perform complete hydrolysis process. Grass species are one of cheap lignocellulosic biomass that can be used as energy source in many bioprocesses and industrial applications. Napier grass, scientific name *Pennisetum purpureum* sp is one of the potential carbon sources, which can be utilized as a substrate for the production of bio-product. The potential of Napier grass as a fermentation substrate was investigated throughout this study.

Isolation and identification of the cellulolytic producing bacteria were carried out by initially searching the bacteria from various environmental sources (cattle waste compost, decayed wood and ponds). The cellulolytic enzymes biosynthesis by the selected bacteria isolate using Napier grass as a substrate in submerged fermentation was carried out in batch cultivation using 250 mL shake-flask. The investigations were followed by statistical optimization using response surface methodology (RSM) approach to obtain the optimized cultural conditions and medium composition for maximum activities of carboxymethyl cellulase (CMCase) and filter paperase (FPase). For the assessment of crude enzyme produced, enzymatic hydrolysis of Napier grass was performed by using untreated and treated (1.4M H_2SO_4 and 7% NaOH) Napier grass to investigate the total reducing sugar production.

A total of twenty- five (25) bacterial isolates were grown on the selective media and only six (6) bacterial isolates showed positive results by exhibiting clear zone on carboxymethyl cellulose (CMC) agar plates, which indicate the enzymes production. The C5/2 isolate was selected as the best cellulolytic enzymes producer due to the highest enzyme activities obtained during the quantitative screening and molecularly identified as *Streptomyces lucitanus* by 16S rDNA sequencing.

The optimum values of the fermentation parameters for CMCase and FPase biosynthesis based on the statistical optimization was determined at initial pH of 7.8, agitation speed 170 rpm, inoculum size 19.7% (v/v) and Napier grass concentration 4.30% (w/v) with maximum activities 7.362 U/mL and 2.895 U/mL, respectively which were close to the predicted values. The production of CMCase and FPase shows improvement, 66% and 30%, respectively by application of the optimum values of parameters during the cultivation. The total reducing sugar produced from enzymatic hydrolysis by using treated Napier grass (1.4M H_2SO_4; 6.541 mg/mL, 7% NaOH; 27.449 mg/mL) as a substrate is higher compared to the untreated Napier grass (1.776 mg/mL).

It can be concluded that the newly isolated *S. lucitanus* C5/2 was able to produce appropriate amount of CMCase and FPase using raw Napier grass as the substrate and significantly improved enzyme activities after statistical optimization by RSM was employed. In addition, the crude cellulolytic enzyme produced by *S. lucitanus* C5/2 has proven to possess potential application in the hydrolysis of lignocellulosic biomass as Napier grass was been used for production of fermentable sugar. From this investigation, Napier grass shows a good fermentation substrate that it can be fully utilized for the production of bio-product.

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

PENGHASIALAN DAN PENGOPTIMUMAN KARBOKSIMETIL SELULASE DAN FILTER PAPERASE OLEH *Streptomyces lucitanus* C5/2 DENGAN MENGGUNAKAN KAEDAH PENAPAIAN SEPARA TENGGELAM RUMPUT NAPIER

Oleh

SHAHIRAH BINTI MANAP

Januari 2017

Pengerusi : Profesor Madya Rosfarizan Mohamad, PhD
Institusi : Perhutanan Tropika dan Produk Hutan

Bahan biojisim lignoselulosa dikenali sebagai bahan mentah yang boleh diperbaharui dan digunakan untuk penghasilan biofuel. Walau bagaimanapun, disebabkan oleh sturuktur molekul selulosa yang kompleks ia menyukarkan proses hidrolisis kepada gula sebagai bahan mentah. Penukaran selulosa kepada gula boleh dimangkinkan oleh sekumpulan enzim dikenali sebagai enzim selulolitik yang dihasilkan oleh mikroorganisma (kulat, bakteria dan protozoa). Enzim selulolitik sistem terdiri daripada endoglukanase, eksoglukanase, dan β-glukosidase di mana setiap enzim ini berinteraksi di antara satu sama lain untuk proses hidrolisis yang sempurna. Spesis rumput merupakan salah satu daripada biojisim lignoselulosa yang murah. Rumput Napier atau nama saintifiknya *Pennisetum purpureum* sp. merupakan salah satu sumber karbon yang berpotensi digunakan sebagai substrat untuk penghasilan bio-produk. Oleh itu, potensi rumput Napier sebagai substrat di dalam proses penapaian dikaji sepanjang kajian ini dijalankan.

Pengasingan dan pengenalpastian bakteria yang boleh menghasilkan enzim selulase telah dijalankan dengan memencilkan bakteria daripada pelbagai sumber alam sekitar (kompos sisa lembu, kayu reput dan kolam). Biosintesis selulase oleh bakteria yang terpilih dengan menggunakan rumput Napier sebagai substrat dalam penapaian separa tenggelam telah dijalankan di dalam kultur sekelompok dengan menggunakan 250 mL kelalang kon. Penyelidikan diikuti dengan pengoptimuman media dan kondisi proses penapaian dengan menggunakan Kaedah Permukaan Gerakbalas (RSM) untuk mendapatkan aktiviti enzim yang maksimum. Seterusnya, untuk penilaian terhadap enzim mentah yang terhasil, hidrolisis enzim telah dijalankan dengan menggunakan rumput Napier yang dirawat dengan 1.4 M H_2SO_4, 7% NaOH dan rumput yang tidak dirawat untuk penghasilan gula.

Hasilnya, sebanyak dua puluh lima (25) bakteria pencilan hidup di atas media terpilih dan hanya enam (6) daripadanya menunjukkan hasil positif dengan menunjukkan zon jelas di atas piring agar karboksimetil selulosa dimana ianya membuktikan penghasilan enzim. Pencilan C5/2 telah dipilih sebagai pengeluar selulase terbaik kerana menghasilkan aktiviti enzim yang tinggi semasa analisis kuantitatif dan dikenal pasti sebagai *Streptomyces lucitanus* oleh penjujukan 16S rDNA.

Nilai-nilai optimum yang diperolehi berdasarkan pengoptimuman statistik untuk biosintesis CMCase dan FPase telah ditentukan pada pH 7.8, kelajuan goncangan 170 rpm, saiz inokulum 19.7% (i/i) and biojisim rumput Napier sebanyak 4.30% (j/i) dengan maksimum aktiviti enzim sebanyak 7.362 U/mL dan 2.895 U/mL, masing masing. Dengan menggunakan nilai parameter yang optimum, penghasilan CMCase dan FPase menunjukkan kadar peningkatan sebanyak 66% dan 30% masing-masing. Jumlah keseluruhan gula yang terhasil melalui proses hidrolisis enzim daripada rumput Napier yang dirawat dengan 1.4 M H_2SO_4 adalah sebanyak 6.541mg/mL and 7% NaOH sebanyak 27.449 mg/mL adalah lebih tinggi berbanding rumput Napier yang tidak dirawat iaitu sebanyak 1.776 mg/mL.

Dapat disimpulkan bahawa *S. lucitanus* C5/2 baru dapat menghasilkan enzim selulolitik dengan menggunakan rumput Napier mentah sebagai substrat dan aktiviti enzim bertambah baik selepas pengoptimuman dengan menggunakan kaedah RSM. Di samping itu, selulase mentah yang dihasilkan oleh *S. lucitanus* C5/2 telah terbukti mempunyai potensi aplikasi dalam hidrolisis biojisim lignoselulosa dimana rumput Napier telah digunakan untuk penghasilan gula mudah menapai. Dari kajian ini, didapati bahawa rumput Napier telah menunjukkan keupayaan sebagai substrat penapaian yang baik dan ia boleh digunakan sepenuhnya untuk penghasilan bio-produk.

ACKNOWLEDGEMENTS

Alhamdulillah, all praise to ALLAH S.W.T who guide my safety, give me strength and endless love. I would like to express a great gratitude to my supervisor, Assoc. Prof. Dr. Rosfarizan Mohamad, who have always had enormous patience and helped me in every way possible. I also would like to express my appreciation from bottom of my heart, to my co-supervisors, especially to Ms. Wan Siti Atikah Wan Omar and not forget to Dr. Wan Zuhainis Saad for their invaluable guidance, constant encouragement and constructive suggestions throughout this study.

My gratitude also goes to my amazing friends and lectures at UiTM and Bioprocess Lab UPM, Sasi, Naidah, Shira, Wawa, Kak.Farina, Abg. Shahrul, Fatehah, Ms. Suhaila, Hj. Badrul and Ms. Shahida, who are directly or indirectly supporting and helping me.

Last but not least, special thanks extended to my family members especially to my beloved father, Hj. Manap bin Omar and mother Pn. Samsiah binti Othman for their invaluable help, enormous support, endless loves, cares, patience and understanding. All prayers from them have brought success to my life.
Thank you for all.

I certify that a Thesis Examination Committee has met on 10 January 2017 to conduct the final examination of Shahirah binti Manap on her thesis entitled "Production and Optimization of Carboxymethyl Cellulase and Filter Paperase of Locally Isolated *Streptomyces lucitanus* C5/2 under Submerged Fermentation of Napier Grass" 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 Master of Science.

Members of the Thesis Examination Committee were as follows:

Luqman Chuah Abdullah, PhD
Professor
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Nor' Aini binti Abdul Rahman, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Awang Ahmad Sallehin Awang Husaini, PhD
Associate Professor
Universiti Malaysia Sarawak
Malaysia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 22 March 2017

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Rosfarizan Mohamad, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Wan Zuhainis Saad, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Wan Siti Atikah Wan Omar
Faculty of Applied Sciences
Universiti Teknologi MARA
(External-Member)

_____
ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of
Chairman of
Supervisory Committee: _____

Signature: _____
Name of
Member of
Supervisory Committee: _____

Signature: _____
Name of
Member of
Supervisory Committee: _____

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xiii
LIST OF FIGURES		xv
LIST OF ABBREVIATIONS		xvi
CHAPTER		
1	**INTRODUCTION**	1
2	**LITERATURE REVIEW**	3
	2.1 Lignocellulosic Biomass	3
	2.1.1 Napier Grass as Potential Biomass Feedstock	4
	2.2 Cellulolytic Enzymes	5
	2.2.1 Cellulolytic Enzymes System	5
	2.2.2 Total Cellulase (Filter paperase) Activity	6
	2.2.3 Carboxymethyl cellulase (Endoglucanase) Activity	6
	2.2.4 Application of Cellulolytic Enzymes in Industry	6
	2.3 Cellulolytic Enzyme Producing Microorganisms.	8
	2.3.1 Fungi	8
	2.3.2 Bacteria and Actinomycetes	8
	2.3.3 *Streptomyces* sp.	8
	2.4 Fermentation Techniques for Cellulolytic Enzyme Production	9
	2.4.1 Submerged Fermentation	9
	2.4.2 Solid State Fermentation	9
	2.5 Factors Influence the Fermentation Process for Cellulolytic Enzymes Production	10
	2.5.1 Initial Medium pH	10
	2.5.2 Agitation Speed	11
	2.5.3 Inoculum Size	11
	2.5.4 Substrate Concentration	11
	2.6 Optimization of Cultural Conditions and Medium Composition	12
	2.7 Lignocellulosic Pre-treatment	13
	2.8 Enzymatic Hydrolysis of Cellulose	14
	2.9 Concluding Remarks	15
3	**MATERIALS AND METHODS**	16
	3.1 Isolation of Cellulolytic Producing Bacteria	16
	3.1.1 Sample Collection	16
	3.1.2 Enrichment Culture and Bacterial Isolation	17
	3.1.3 Morphological Studies	17
	3.1.4 Biochemical Test	17

3.1.5 Qualitative Screening of Cellulolytic Enzymes Activity 18
3.1.6 Quantitative Screening of Cellulolytic Enzymes Activity 18
3.1.7 Genomic DNA Extraction 18
3.1.8 Identification by 16S rDNA Sequencing Analysis 18
3.1.9 Phylogenetic Analysis 19
3.1.10 Culture Maintenance 19
3.2 Characterization of Napier Grass 19
3.2.1 Sample Preparation 19
3.2.2 Moisture and Total Solid Content 20
3.2.3 Fiber Extraction 20
3.2.4 Determination of Lignin Content 20
3.2.5 Determination of Holocellulose Content 21
3.2.6 Determination of α-cellulose Content 22
3.2.7 Determination of Ash Content 22
3.2.8 Determination of Nutrients Content 23
3.3 Cellulolytic Enzymes Biosynthesis using Submerged Fermentation of Napier Grass 23
3.3.1 Inoculum Preparation 23
3.3.2 Submerged Fermentation 23
3.4 Medium Composition and Culture Conditions Optimization for Cellulolytic Enzymes Biosynthesis using Statistical Approach 24
3.4.1 Full Factorial Experimental Design 24
3.4.2 Central Composite Design of RSM 24
3.5 Enzymatic Hydrolysis of Napier Grass 25
3.5.1 Preparation of Crude Cellulolytic Enzymes 25
3.5.2 Alkaline Pre-treatment 25
3.5.3 Acid Pre-treatment 25
3.5.4 Enzymatic Hydrolysis of Napier Grass 25
3.6 Analytical Procedure 26
3.6.1 Determination of Cell Concentration 26
3.6.2 Determination of Filter paperase 26
3.6.3 Determination of Carboxymethyl Cellulase 27
3.6.4 Determination of Total Protein Activity 27
3.6.5 Reducing Sugar Analysis 27
3.7 General Experimental Design 28

4 RESULTS AND DISCUSSION 30
4.1 Isolation of Cellulolytic Enzymes Producing Bacteria 30
4.1.1 Preliminary Screening 30
4.1.2 Morphological Characteristics and Biochemical Properties 32
4.1.3 Quantitative Screening of CMCase and FPase Production 34
4.1.4 Comparison of Cellulolytic Enzymes Production 37
4.1.5 Identification of Bacteria by 16S rDNA Sequencing 39
4.2 Chemical Compositions of Napier Grass 40
4.3 Cellulolytic Enzymes Production by Submerged Fermentation of Napier Grass 42

4.3.1 Carboxymethyl Cellulase and Filter-paperase Activities 42
4.3.2 Growth Profile of *S. lucitanus* C5/2 43
4.4 Optimization of Culture Conditions and Medium Composition using Statistical Approach 44
4.4.1 Screening of significant factors by 2^4 Full Factorial Design 44
4.4.2 Central Composite design 46
4.4.3 Verification of Experimental Design 55
4.5 Evaluation of Crude Cellulolytic Enzymes for Hydrolysis of Napier Grass 56

5 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK 58

REFERENCES 60
APPENDICES 68
BIODATA OF STUDENT 75
LIST OF PUBLICATIONS 76

LIST OF TABLES

Table		Page
2.1	The comparison of Napier grass composition with other lignocellulosic biomass materials.	4
2.2	The application of cellulase in various industries.	7
2.3	List of references on both SmF and SSF reported using various microorganisms	10
2.4	List of cellulolytic enzymes production by using RSM study	12
2.5	Advantages and disadvantages of pre-treatment methods for lignocellulose hydrolysis.	14
3.1	Type of samples and source location	16
3.2	Enrichment media compositions	17
3.3	Broth Medium Compositions	18
3.4	Parameters and coded values of FFD	24
3.5	Reagents composition of Protein Assay Lowry kit (Nacalai Tesque,INC.)	27
4.1	Clear zone diameters of cellulolytic enzyme producing bacteria isolated from different sources.	31
4.2	The morphological characteristics and biochemical properties of the six positive isolates.	33
4.3A	Comparison of the performance of bacteria isolates cultivation in shake-flask using CMC as a substrate for FPase production.	38
4.3B	Comparison of the performance of bacteria isolates cultivation using CMC as a substrate for CMCase production.	38
4.3C	The growth performance of bacteria isolates cultivation.	38
4.4	Estimation of the chemical compositions of stem and leaf part of Napier grass	41
4.5	Comparison of CMCase and FPase activities by *S. lucitanus* C5/2 using different substrates	43
4.6	The 2^4 full factorial design and response for screening experiments.	45
4.7	ANOVA and regression analysis results of the first-order model for 2^4 full factorial designs.	46
4.8	Parameters and their coded levels for the central composite design used for CMCase and FPase production by *S. lucitanus* C5/2.	47
4.9	CCD experimental design for cellulase production by *S. lucitanus* C5/2.	48
4.10	ANOVA and regression analysis for response surface quadratic model	49

4.11 The verification of experimental and predicted values for CMCase and FPase under optimized conditions 56

4.12 The comparison of CMCase and FPase activities between optimized and non-optimized conditions 56

4.13 The chemical composition of untreated and treated Napier grass and total fermentable sugar produced during hydrolysis by using crude cellulase synthesis by *S. lucitanus* C5/2. 57

LIST OF FIGURES

Figure		Page
2.1	Mechanisms of cellulase action	5
2.2	Effect of pre-treatment on accessibility for enzymatic hydrolysis	13
3.1	Napier grass	20
3.2	The Soxhlet apparatus for fibre extraction	21
3.3	General flow of the experimental design	28
4.1	Clear zone formation of the six positive isolates on CMC agar plate	31
4.2	FPase activity (U/mL) profiles of bacteria isolates cultivation using CMC as a substrate in shake-flask culture.	35
4.3	CMCase activity (U/mL) profiles of bacteria isolates cultivation using CMC as a substrate in shake-flask culture.	35
4.4	Total protein activity (mg/mL) profiles of bacteria isolates cultivation using CMC as a substrate in shake-flask culture.	36
4.5	The growth profile of bacteria isolates cultivation using CMC as a substrate in shake-flask	37
4.6	The phylogenetic tree – Neighbor Joining	40
4.7	Submerged fermentation of Napier grass by *S. lucitanus* C5/2	42
4.8	Response surface 3D plot for CMCase (U/mL) production by *S. lucitanus* C5/2	50
4.9	Response surface 3D plot for FPase (U/mL) production by *S. lucitanus* C5/2	54

LIST OF ABBREVIATIONS

CCD	Central Composite Design
CMC	Carboxymethyl Cellulose
CMCase	Carboxymethyl Cellulase
DCW	Dry Cell Weight
DNS	Dinitrosalicylic acid
FFD	Full Factorial Design
FPase	Filter Paperase
NCBI	National Centre of Biotechnology Information
NIRS	Near Infrared Reflectance Spectrophotometer
RSM	Response Surface Methodology
SmF	Submerged Fermentation
SSF	Solid State Fermentation
TAPPI	Technical Association of Pulp and Paper Industry
TS	Total Solid

CHAPTER 1

INTRODUCTION

Lignocellulosic biomass is currently of interest by many industries and researchers to use as a prime source for fermentable sugar production due to most abundantly available and cheap feedstock. The lignocellulose consists of three main components: cellulose, hemicellulose, and lignin, which build up by a chain of sugar molecules. These chains can be hydrolyzed to produce monomeric sugars. The bioconversion of cellulose to sugar can be catalyzed by a group of enzymes called cellulolytic enzymes that mainly produced by microorganisms (fungi and bacteria). A cellulolytic enzymes system comprises of at least three main components; endoglucanase (1,4-β-D-glucan-4-glucanohyrolase, carboxymethyl cellulase), exoglucanase (1,4-β-D-glucan-glucanohydrolase, avicelase) and β-glucosidase (Dashtban et al., 2010; Demain et al., 2005). These three components work together for a complete enzymatic hydrolysis. The endoglucanase responsible for randomly hydrolyze the β-1,4 bonds in cellulose molecules, while exoglucanase helps to release the non- reducing end of a cellulose chain and lastly β-glucosidase hydrolyses cellobiose to glucose.

For many years, numerous cellulolytic producing microorganisms have been isolated and identified by researchers (Gupta et al., 2012; Huang et al., 2012; Kim et al., 2012; Liang et al., 2014). Many studies have put more interest on fungi because it can produce cellulolytic enzymes abundantly and easy to extract (Maki et al., 2009). However, in recent years, researchers have more interest on the isolation and characterization of cellulolytic enzymes producing bacteria in order to obtain more effective cellulolytic enzymes producing bacteria from various sources (Doi, 2008). Researchers have more focus on bacteria because of their fast growth, expression of multienzyme complexes and resistance to extreme environment (Liang et al., 2014).

Grass species are one of the cheap lignocellulosic biomass where people have not realized the importance of them as a carbon source for a bio-product production. As a lignocellulosic perennial crop, the potential of conversion to energy in the form of biofuels and another by-product is highly promising. Recent research shows some interests to use perennial grasses such as switchgrass and ryegrass as substrates for bioethanol production (Liong et al., 2012). Napier grass (*Pennisetum purpureum* sp.) is known as a C4 perennial grasses that currently used as the feedstock for cow and cattle farming. However, the knowledge about this grass as an energy source is limited. The potential of Napier grass as a substrate for cellulolytic enzymes production by bacterium was investigated throughout this study.

The production of cellulolytic enzymes from Napier grass using fermentation process by bacterial strain could bring an economic impact to an industry especially in bio-ethanol industry. For bio-ethanol production, cellulolytic enzymes are widely used as a hydrolytic enzyme to break down cellulose into fermentable sugar. Therefore, the production of cellulolytic enzymes in large quantity is highly demanding.

The optimization of the medium compositions and fermentation conditions is an important step in producing a maximum production of the enzyme. The optimization by statistical approach using response surface methodology (RSM) gives an alternative technique to analyze optimized conditions and enable the researcher to design the experiment, building models, and evaluate the effect of factors and response through the investigation (Hao et al., 2006; Norfarina et al., 2010). Response surface methodology (RSM) approach has been successfully applied to optimize the medium compositions and fermentation conditions for the cellulolytic enzyme production using fungi by submerged fermentation (Anuradha et al., 2012; Carvalho et al., 2014).

The scope of this study was focused on the improvement of cellulolytic enzymes biosynthesis by locally isolated bacterium using Napier grass as a substrate. The Napier grass hydrolysate containing fermentable sugar was also prepared using crude cellulolytic enzyme obtained from the bacterium cultivation using Napier grass as the substrate. Hence, the objectives of this study are:

i. To screen, isolate and identify the cellulolytic enzymes producing bacteria from various sources.
ii. To characterize the Napier grass as potential substrate for cellulolytic enzymes production.
iii. To optimize the medium composition and culture conditions for the biosynthesis of carboxymethyl cellulase and filter paperase by the isolated bacterium using statistical approach.
iv. To evaluate the performance of Napier grass hydrolysis using crude cellulolytic enzyme for fermentable sugar production.

REFERENCES

Abou-Taleb, K. A. A., Mashhoor, W. A., Nasr, S. A., Sharaf, M. S., & Abdel-Azeem, H. H. (2009). Nutritional and Environmental Factors Affecting Cellulase Production by Two Strains of Cellulolytic *Bacilli*. *Australian Journal of Basic and Applied Sciences*, *3*(February), 2429–2436.

Abubakar, F. A., & Oloyede, O. B. (2013). Production and Activity of Cellulase from *Aspergillus niger* Using Rice Bran and Orange Peel as Substrates. *International Journal of Scientific Research and Management*, *1*(5), 285–291.

Acharya, S., & Chaudhary, A. (2012). Optimization of Fermentation Conditions for Cellulases Production by *Bacillus licheniformis* MVS1 and *Bacillus* sp. MVS3 Isolated from Indian Hot Spring. *Brazilian Archives of Biology and Technology*, *55*, 497–503.

Aganga, A. A., Omphile, U. J., Thema, T., & Baitshotlhi, J. C. (2005). Chemical Composition of Napier Grass (*Pennisetum purpureum*) at Different Stages of Growth and Napier Grass Silages with Additives. *Journal of Biological Sciences*, *5*, 493–496.

Agrawal, S. (2015). Solid State Fermentation for Cellulase Production. *Biotechnology Research Journal*, *1*(1), 109–112.

Alam, M. Z., Muyibi, S. a., & Wahid, R. (2008). Statistical Optimization of Process Conditions for Cellulase Production by Liquid State Bioconversion of Domestic Wastewater Sludge. *Bioresource Technology*, *99*(11), 4709–4716.

Alvira, P., Tomás-Pejó, E., Ballesteros, M., & Negro, M. J. (2010). Pretreatment Technologies for an Efficient Bioethanol Production Process Based on Enzymatic Hydrolysis: A Review. *Bioresource Technology*, *101*(13), 4851–4861.

Anuradha Jabasingh, S., & Valli Nachiyar, C. (2012). Optimization of Cellulase Synthesis by RSM and Evaluation of Ethanol Production from Enzymatically Hydrolyzed Sugarcane Bagasse Using *Saccharomyces cerevisiae*. *Journal of Scientific and Industrial Research*, *71*(5), 353–359.

Atika, N., Remli, M., Mohamad, R., & Abd-aziz, S. (2014). Effects of Chemical and Thermal Pretreatments on the Enzymatic Saccharification of Rice Straw for Sugars Production, *Bioresources*, *9*(Moa 2004), 510–522.

Bin Amwarali Khan, F. A., & Husaini, A. A. S. A. (2006). Enhancing α– amylase and Cellulase in vivo Enzyme Expressions on Sago Pith Residue using *Bacillus amyloliquefaciens* UMAS 1002. *Biotechnology*, *5*(3), 391– 403.

Binod, P., Janu, K. U., Sindhu, R., & Pandey, A. (2011). *Biofuels: Alternative Feedstocks and Conversion Processes. Biofuels* (1st ed.) Burlington: Elsevier Inc.

Bui, H. B. (2014). Isolation of Cellulolytic Bacteria, Including actinomycetes, from Coffee Exocarps In Coffee-Producing Areas in Vietnam. *International Journal of Recycling of Organic Waste in Agriculture*, *3*(48).

Carvalho, M. L. D. A. De, Carvalhok, D. F., Gomes, E. D. Ba. G., Maeda, R. N., Santa Anna, L. M. M., Castro, A. M. De, & Pereira Jr, N. (2014). Optimisation of Cellulase Production by *Penicillium funiculosum* in a Stirred Tank Bioreactor Using Multivariate Response Surface Analysis. *Enzyme Research*, *2014*, 8.

Chellapandi, P., & Jani, H. M. (2008). Production of Endoglucanase by the Native Strains of *Streptomyces* Isolates in Submerged Fermentation. *Brazilian Journal of Microbiology*, *39*(1), 122–127.

Cheng, J. J., & Timilsina, G. R. (2011). Status and Barriers of Advanced Biofuel Technologies: A Review. *Renewable Energy*, *36*(12), 3541–3549.

Chu, F. J., Lin, C. W., I, Y. P., Wu, C. H., & Chen, D. H. (2012). Hydrolysis of Bamboo Cellulose and Cellulase Characteristics by *Streptomyces griseoaurantiacus* ZQBC691. *Journal of the Taiwan Institute of Chemical Engineers*, *43*(2), 220–225.

Conde-Mejía, C., Jiménez-Gutiérrez, A., & El-Halwagi, M. (2012). A Comparison of Pretreatment Methods for Bioethanol Production from Lignocellulosic Materials. *Process Safety and Environmental Protection*, *90*(3), 189–202.

Das, A., Bhattacharya, S., & Murali, L. (2010). Production of Cellulase from a Thermophilic *Bacillus sp*. Isolated from Cow Dung. *American-Eurasian Journal of Agricultural & Environmental Sciences*, *8*(6), 685–691.

Dashtban, M., Maki, M., Leung, K. T., Mao, C., & Qin, W. (2010). Cellulase Activities in Biomass Conversion: Measurement Methods and Comparison. *Critical Reviews in Biotechnology*, *30*(4), 302–309.

Deka, D., Das, S. P., Sahoo, N., Das, D., Jawed, M., Goyal, D., & Goyal, A. (2013). Enhanced Cellulase Production from *Bacillus subtilis* by Optimizing Physical Parameters for Bioethanol Production, *ISRN Biotechnology*, *2013*.

Demain, A. L., Newcomb, M., Wu, J. H. D., (2005). Cellulase, Clostridia, and Ethanol, *Microbiology and Molecular Biology Review*, *69*(1).

Doi, R. H. (2008). Cellulase of Mesophilic Microbes: Cellulosome and Non-cellulosome Producer. *Annals of the New York Academy of Sciences*, *1125*, 267-279.

Dos Santos, T. C., Gomes, D. P. P., Bonomo, R. C. F., & Franco, M. (2012). Optimisation of Solid State Fermentation of Potato Peel for the Production of Cellulolytic Enzymes. *Food Chemistry*, *133*(4), 1299–1304.

Ebringerova, A., Hromadkova, Z., & Heinze, T. (2005). Hemicellulose. *Advance in Polymer Science*, *186*, 1–67.

El-sersy, N. A., Abd-elnaby, H., Abou-elela, G. M., Ibrahim, H. A. H., & El-toukhy, N. M. K. (2010). Optimization, Economization and Characterization of Cellulase Produced by Marine *Streptomyces ruber*. *African Journal of Biotechnology*, *9*(38), 6355–6364.

Franco-Cirigliano, M. N., Rezende, R. D. C., Gravina-Oliveira, M. P., Pereira, P. H. F., do Nascimento, R. P., Bon, E. P. D. S., Macrae, A., Coelho, R. R. R. (2013). *Streptomyces misionensis* PESB-25 Produces a Thermoacidophilic Endoglucanase Using Sugarcane Bagasse and Corn Steep Liquor as the Sole Organic Substrates. *BioMed Research International*, *2013*.

Ghose, T. K. (1987). Measurement of Cellulase Activity, *Pure and Applied Chemistry*, *59*, 257–268.

Gunny, A. A. N., Arbain, D., Jamal, P., & Gumba, R. E. (2014). Improvement of Halophilic Cellulase Production from Locally Isolated Fungal Strain. *Saudi Journal of Biological Sciences*, *22*(4), 476–483.

Gupta, P., Samant, K., & Sahu, A. (2012). Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential. *International Journal of Microbiology*, *2012*.

Gusakov, A., & Sinitsyn, A. (2012). Cellulases from *Penicillium* Species for Producing Fuels from Biomass. *Biofuels*, *3*(4), 463–477.

Hao, X., Yu, X., & Yan, Z.-L. (2006). Optimization of the Medium for the Production of Cellulase by the Mutant *Trichoderma reesei* WX-112 Using Response Surface Methodology. *Food Technology and Biotechnology*, *44*(1), 89–94.

Huang, S., Sheng, P., & Zhang, H. (2012). Isolation and Identification of Cellulolytic Bacteria from the Gut of *Holotrichia parallela* Larvae (Coleoptera: Scarabaeidae). *International Journal of Molecular Sciences*, *13*(3), 2563–2577.

Immanuel, G., Dhanusha, R., Prema, P., & Palavesam, A. (2006). Effect of Different Growth Parameters on Endoglucanase Enzyme Activity by Bacteria Isolated from Coir Retting Effluents of Estuarine Environment. *International Journal of Environmental Science and Technology*, *3*, 25–34.

Irfan, M., Safdar, A., Syed, Q., & Nadeem, M. (2012). Isolation and Screening of Cellulolytic Bacteria from Soil and Optimization of Cellulase Production and Activity. *Turkish Journal of Biochemistry*, *37*(3), 287–293.

Jaradat, Z., Dawagreh, A., Ababneh, Q., & Saadoun, I. (2008). Influence of Culture Conditions on Cellulase Production by *Streptomyces* sp. (Strain J2). *Jordan Journal of Biological Sciences*, *1*(4), 141–146.

Juturu, V., & Wu, J. (2014). Microbial Cellulases: Engineering, Production and Applications. *Renewable and Sustainable Energy Reviews*, *22*, 188-203.

Karmakar, M., & Ray, R. R. (2013). Endoglucanase Synthesis from Submerged Fermentation of Dried Grass by *Streptomyces* sp. *American Journal of Bioengineering and Biotechnology*, *2013*(1), 1–10.

Khalilah, A. K., Shuhaimi, M., Rosfarizan, M., Arbakariya, A., Yamin, S., Yazid, A. M., Siti Aqlima, A., & Farrah Aini, D. (2014). Optimization of Milk-Based Medium for Efficient Cultivation of *Bifidobacterium pseudocatenulatum* G4 Using Face-Centered Central Composite-Response Surface Methodology. *BioMed Research International*, *2014*.

Khare, A., & Upadhyay, R. S. (2011). Influence of Some Cultural Factors on Production of Cellulase and β-1, 3-glucanase by the Mutant Strains of *Trichoderma viride*. *Journal of Agriculture Technology*, *7*(2), 403–412.

Kim, Y.K., Lee, S.C., Cho, Y.Y., Oh, H.J., & Ko, Y. H. (2012). Isolation of Cellulolytic *Bacillus subtilis* Strains from Agricultural Environments. *ISRN Microbiology*, *2012*, 1–9.

Kroothdilaganandh, J. (2000). Isolation and Selection of Thermotolerant Bacteria Capable of Producing Cellulose. *Chiang Mai University Press, Chiang Mai.*

Kuhad, R. C., Gupta, R., & Singh, A. (2011). Microbial Cellulases and Their Industrial Applications. *Enzyme Research*, *2011*, 1–10.

Lee, M. K., Tsai, W. T., Tsai, Y. L., & Lin, S. H. (2010). Pyrolysis of napier grass in an Induction-heating Reactor. *Journal of Analytical and Applied Pyrolysis*, *88*(2), 110–116.

Lee, H. V, Hamid, S. B. A., & Zain, S. K. (2014). Conversion of Lignocellulosic Biomass to Nanocellulose: Structure and Chemical Process. *The Scientific World Journal*, *2014*, 1–22.

Li, Y., Lin, J., Meng, D., Lu, J., Gu, G., & Mao, Z. (2006). Effect of pH, Cultivation Time and Substrate Concentration on the Endoxylanase Production by *Aspergillus awamori* ZH-26 under Submerged Fermentation Using Central Composite Rotary Design. *Food Technology and Biotechnology*, *44*(4), 473–477.

Liang, Y., Zhang, Z., Wu, M., Wu, Y., & Feng, J. (2014). Isolation , Screening , and Identification of Cellulolytic Bacteria from Natural Reserves in the Subtropical Region of China and Optimization of Cellulase Production by *Paenibacillus terrae* ME27-1. *BioMed Research International*, *2014*.

Limayem, A., & Ricke, S. C. (2012). Lignocellulosic Biomass for Bioethanol Production: Current Perspectives, Potential Issues and Future Prospects. *Progress in Energy and Combustion Science*, *38*(4), 449–467.

Liong, Y. Y., Halis, R., Lai, O. M., & Mohamed, R. (2012). Conversion of Lignocellulosic Biomass from Grass to Bioethanol Using Materials Pretreated with Alkali and the White Rot Fungus, *Phanerochaete chrysosporium*. *BioResources*, *7*(4), 5500–5513.

Lowry, O. H., Roserbough, N. J., Randall, R. J., & Lewis, A. (1951). Protein Measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry*, *193*, 265-275.

Maki, M., Leung, K. T., & Qin, W. (2009). The Prospects of Cellulase-producing Bacteria for the Bioconversion of Lignocellulosic Biomass. *International Journal of Biological Sciences*, *5*(5), 500-516.

Mandels, M., Andreotti, R., Roche, C. (1976). Measurement of saccharifying cellulase. *Biotechnology Bioengineering Symposium*, 21-33.

Margeot, A., Hahn-Hagerdal, B., Edlund, M., Slade, R., & Monot, F. (2009). New Improvements for Lignocellulosic Ethanol. *Current Opinion in Biotechnology*, *20*(3), 372–80.

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, *31*, 426–428.

Mohammed, I. Y., Abakr, Y. A., Kazi, F. K., Yusup, S., Alshareef, I., & Chin, S. A. (2015). Comprehensive Characterization of Napier Grass as a Feedstock for Thermochemical Conversion. *Energies*, *8*, 3403–3417.

Narendhirakannan, R. T., Rathore, S. S., & Mannivannan, A. (2014). Screening of Cellulase Producing Microorganisms from Lake Area Containing Water Hayacinth for Enzymatic Hydrolysis of Cellulose. *Journal of Advanced Scientific Research*, *5*(3), 23–30.

Norfarina, M. N., Rosfarizan, M., Hooi Ling, F., & Raha, A. R. (2010). Improvement of Folate Biosynthesis by Lactic Acid Bacteria Using Response Surface Methodology. *Food Technology and Biotechnology*, *48*(February), 243–250.

Pirzadah, T., Garg, S., Singh, J., Vyas, A., Kumar, M., Gaur, N., Kumar, V., Kumar, M. (2014). Characterization of Actinomycetes and Trichoderma spp. for Cellulase Production Utilizing Crude Substrates by Response Surface Methodology. *SpringerPlus*, *3*(1), 622.

Prasad, P., Singh, T., & Bedi, S. (2013). Characterization of the Cellulolytic Enzyme Produced by *Streptomyces griseorubens* (Accession No. AB184139) Isolated from Indian soil. *Journal of King Saud University - Science*, *25*(3), 245–250.

Ray, A. K., Bairagi, A., Ghosh, K. S., & Sen, S. K. (2007). Optimization of Fermentation Conditions for Cellulase Production by *Bacillus subtilis* CY5 and *Bacillus circulans* TP3 Isolated from Fish Gut. *International Journal for Ichthyology and Fisheries*, *37*, 47–53.

Reddy, K. O., Maheswari, C. U., Reddy, D. J. P., & Rajulu, A. V. (2009). Thermal properties of Napier grass fibers. *Materials Letters*, *63*(27), 2390–2392.

Reddy, K. O., Maheswari, C. U., Shukla, M., & Rajulu, a. V. (2012). Chemical Composition and Structural Characterization of Napier Grass Fibers. *Materials Letters*, *67*(1), 35–38.

Sadhu, S., & Maiti, T. K. (2013). Cellulase Production by Bacteria : A Review. *British Microbiology Research Journal*, *3*(3), 235–258.

Sadouk-Hachaïchi, Z., Tazerouti, A., & Hacene, H. (2014). Growth Kinetics Study of a Bacterial Consortium Producing Biosurfactants, Constructed with Six Strains Isolated from an Oily Sludge. *Advances in Bioscience and Biotechnology*, *5*, 418–425.

Samira, M., Mohammad, R., & Gholamreza, G. (2011). Carboxymethyl-cellulase and Filter-paperase Activity of New Strains Isolated from Persian Gulf. *Microbiology Journal*, *1*(1), 8–16.

Sara, S., & Raj, B. (2011). Ethanol Production From Lignocellulosic Biomass of Energy Cane. *International Biodeterioration and Biodegradation*, *65*(1), 142–146.

Sebolai, T. M., Aganga, A. A., Nsinamwa, M., & Moreki, J. C. (2012). Effects of Different Silage Preservatives on Silage Quality of *Pennesitum purpureum* Harvested at Different Harvesting Periods. *Online Journal of Animal and Feed Research*, *2*(2), 139–144.

Sethi, S., Datta, A., Gupta, B. L., & Gupta, S. (2013). Optimization of Cellulase Production from Bacteria Isolated from Soil. *ISRN Biotechnology*, *2013*.

Shahida Hanum, K., Marlina, M. M., Wan Siti Atikah, W. O., Siti Suhaila, H., Badrul HIsham, M. N., Nurul Zaimatul Akma, A., Shahirah, M., & Rosfarizan, M. (2015). Surface Morphology and Chemical Composition of Napier Grass Fiber. *Malaysian Journal of Analytical Sciences*, *19*(4), 889–895.

Shankar, T., & Isaiarasu, L. (2012). Statistical Optimization for Cellulase Production by *Bacillus pumilus* EWBCM1 Using Response Surface Methodology. *Global Journal of Biotechnology & Biochemistry*, *7*(1), 1–6.

Silverstein, R. A., Chen, Y., Sharma-Shivappa, R.R. Boyette, M. D., & Osborne, J. (2007). A Comparison of Chemical Pretreatment Methods for Improving Saccharification of Cotton Stalks. *Bioresource Technology*, *98*, 3000–3011.

Singh, J., & Kaur, P. (2012). Optimization of Process Parameters for Cellulase Production from *Bacillus sp* . JS14 in Solid Substrate Fermentation Using Response Surface Methodology. *Brazilian Archives of Biology and Technology*, *55*(August), 505–512.

Singh, S., Du Preez, J., Pillay, B., & Prior, B. A. (2000). The Production of Hemicellulases by *Thermomyces lanuginosus* Strain SSBP: Influence of Agitation and Dissolved Oxygen Tension. *Applied Microbiology and Biotechnology*, *54*(5), 698–704.

Singh, S., Pranaw, K., Singh, B., Tiwari, R., & Nain, L. (2014). Production, Optimization and Evaluation of Multicomponent Holocellulase Produced by *Streptomyces sp*. ssr-198. *Journal of the Taiwan Institute of Chemical Engineers*, *45*(5), 2379–2386.

Stubbs, T. L., Kennedy, A. C., & Fortuna, A. M. (2010). Using NIRS to Predict Fiber and Nutrient Content of Dryland Cereal Cultivars. *Journal of Agricultural and Food Chemistry*, *58*(1), 398–403.

Subramaniyam, R., & Vimala, R. (2012). Solid State and Submerged Fermentation for the Production of Bioactive Substances: A Comparative Study. *International Journal Science Nature*, *3*(3), 480–486.

Sukumaran, R. K., Singhania, R. R., & Pandey, A. (2005). Microbial Cellulases - Production, Applications and Challenges. *Journal of Scientific and Industrial Research*, *64*(11), 832–844.

Sun, Y., & Chen, J. (2002). Hydrolysis of Lignocellulosic Materials for Ethanol Production. A Review. *Bioresource Technology*, *88*, 1–11.

Taherzadeh, M. J., & Karimi, K. (2008). Pretreatment of lignocellulosic wastes to Improve Ethanol and Biogas Production: A Review. *International Journal of Molecular Sciences*, *9*, 1621-1651.

TAPPI. (2000). TAPPI Test Methods 2000-2001 Technical Association of the Pulp and Paper Industry, Atlanta USA.

Techapun, C., Poosaran, N., Watanabe, M., & Sasaki, K. (2003). Optimization of Aeration and Agitation Rates to Improve Cellulase-free Xylanase Production by Thermotolerant *Streptomyces* sp. Ab106 and Repeated Fed-Batch Cultivation Using Agricultural Waste. *Journal of Bioscience and Bioengineering*, *95*(3), 298–301.

Tuncer, M., Kuru, A., Isikli, M., Sahin, N., & Çelenk, F. G. (2004). Optimization of Extracellular Endoxylanase, Endoglucanase and Peroxidase Production by *Streptomyces* sp. F2621 Isolated in Turkey. *Journal of Applied Microbiology*, *97*(4), 783–791.

Waghmare, P. R., Kshirsagar, S. D., Saratale, R. G., Govindwar, S. P., & Saratale, G. D. (2014). Production and Characterization of Cellulolytic Enzymes by Isolated *Klebsiella* sp. prw-1 Using Agricultural Waste Biomass. *Emirates Journal of Food and Agriculture*, *26*(1), 44–59.

Wang, Z., & Cheng, J. J. (2011). Lime Pretreatment of Coastal Bermudagrass for Bioethanol Production. *Energy and Fuels*, *25*(4), 1830–1836.

Yang, B., Dai, Z., Ding, S.-Y., & Wyman, C. E. (2011). Enzymatic Hydrolysis of Cellulosic Biomass. *Biofuels*, *2*(4), 421–450.

Zhang, X., & Zhang, Y. P. (2013). Cellulases: Characteristics, Sources, Production, and Applications. In T. Y. Shang, A. E. E. Hesham, & T. Nuttha (Eds.), *Bioprocessing Technologies in Biorefinery for Sustainable Product of Fuels, Chemicals, and Polymers*, 1st ed., 131–146. John Wiley & Sons, Inc.

Zhang, Y. H. P., Hong, J., & Ye, X. H., (2009). Cellulase Assay. Jonathan, R. M (Ed.), *Biofuels: Methods and Protocols, Method in Molecular Biology, 581*, 213-231. Springer Science.

Zhang, Y. H. P., Himmel, M. E., & Mielenz, J. R. (2006). Outlook of Cellulase Improvement: Screening and Selection Strategies. *Biotechnology Advances, 24*, 452–481.

Zoppas, F. M., Meneguzzi, Á., & Tramontina, F. (2013). Alternatives for Cellulase Production in Submerged Fermentation with Agroindustrial Wastes. *International Journal of Modern Engineering Research*, *3*(4), 2374–2381.

BIODATA OF STUDENT

The student, Shahirah binti Manap was born on February 11th, 1987 in Temerloh Pahang. She received her early primary education at Sekolah Kebangsaan Paya Pulai, Temerloh Pahang before studying at Sekolah Menengah Kebangsaan Temerloh, Pahang for secondary education. In 2005, she enrolled her study at Universiti Teknologi Mara, Pahang Campus as a Diploma student in Sciences for three years. For the following year, she was promoted to further her study in bachelor degree at UiTM, Shah Alam Campus and graduated with Honours Degree of Biomolecular Science in 2011.

After graduated, she works as a sales assistant at YHC Berkat Pharmacy Sdn Bhd. for one year. At 2013, she decided to further her study to a master level and joined UiTM, Jengka Pahang as a research assistant for six months. In the same year, she was supported to further her study in Master Degree at UPM by financial funding of MyBrain15 and the Exploratory Research Grant Scheme (ERGS) 600-RMI/ERGS 5/3 (14/2012).

LIST OF PUBLICATIONS

Shahirah, M., Wan Zuhainis, S., Wan Siti Atikah, W.O., Siti Suhaila, H., and Rosfarizan, M. (2014). Cellulolytic Enzyme Producing Bacteria for Biological Pretreatment of Lignocelulose. National Postgraduate Seminar (10th September 2014), Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia.

Shahirah, M., Wan Zuhainis, S., Wan Siti Atikah,W.O., Siti Suhaila, H., and Rosfarizan, M. (2014). Carboxymethyl- cellulase and Filter paperase Activity of Newly Isolated Strains. 32nd Symposium of Malaysian Society for Microbiology (6-8 December 2014), Terengganu Equestrian Resort, Malaysia.

Shahida Hanum, K., Marlina, M. M., Wan Siti Atikah, W. O., Siti Suhaila, H., Badrul Hisham, M. N., Nurul Zaimatul Akma, A., Shahirah, M., Rosfarizan, M. (2015). Surface Morphology and Chemical Composition of Napier Grass Fiber. Malaysian Journal of Analytical Sciences, 19(4), 889–895.

Shahirah, M., Rosfarizan, M., Wan Zuhainis, S., Siti Suhaila, H., Marlina, M. M., Shahida Hanum, K., Badrul Hisham, M. N. (2016). Optimization of Medium Composition and Culture Conditions of Filter-paperase by UPMC1106 using *Pennisetum purpureum* as a Substrate. Chemical Engineering Transection Vol.52 (Accepted).

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : _____

TITLE OF THESIS / PROJECT REPORT :

PRODUCTION AND OPTIMIZATION OF CARBOXYMETHYL CELLULASE AND FILTER PAPERASE OF LOCALLY ISOLATED *Streptomyces lucitanus* C5/2 UNDER SUBMERGED FERMENTATION OF NAPIER GRASS

NAME OF STUDENT: SHAHIRAH BINTI MANAP

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from_____ until _____ (date) (date)

Approved by:

_____	_____
(Signature of Student)	(Signature of Chairman of Supervisory Committee)
New IC No/ Passport No.:	Name:
Date :	Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]