GLUCOSE PRODUCTION FROM CORNCOB BY MICROBIAL HYDROLYSIS USING BACTERIA ISOLATED FROM THAI HIGHER TERMITES

Nattawut Hokittikul

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science The Petroleum and Petrochemical College, Chulalongkorn University in Academic Partnership with The University of Michigan, The University of Oklahoma, Case Western Reserve University, and Institut Français du Pétrole 2013

Thesis Title:	Glucose Production from Corncob by Microbial Hydrolysis
	Using Bacteria Isolated from Thai Higher Termites
By:	Nattawut Hokittikul
Program:	Petrochemical Technology
Thesis Advisors:	Assoc. Prof. Pramoch Rangsunvigit
	Prof. Sumaeth Chavadej

Accepted by the Petroleum and Petrochemical College, Chulalongkorn University, in partial fulfilment of the requirements for the Degree of Master of Science.

...... College Dean

(Asst. Prof. Pomthong Malakul)

Thesis Committee:

hannoch Q

(Assoc. Prof. Pramoch Rangsunvigit)

Sumaeth ander.

(Prof. Sumaeth Chavadej)

 $\neg \checkmark$

(Asst. Prof. Siriporn Jongpatiwut)

send 2

(Prof. Suntud Sirianuntapiboon)

ABSTRACT

5471009063: Petrochemical Technology Program
Nattawut Hokittikul: Glucose Production from Corncob by
Microbial Hydrolysis Using Bacteria Isolated from Thai Higher
Termites
Thesis Advisors: Assoc. Prof. Pramoch Rangsunvigit and Prof.
Sumaeth Chavadej 65 pp.
Keywords: Microbial hydrolysis; Glucose production; *Bacillus subtilis*;
Corncob

Microbial hydrolysis of corncob to glucose by cellulase-producing bacteria (*Bacillus subtilis*) isolated from Thai higher termites, *Microcerotermes* sp., was investigated. Corncob consists of 43.82% cellulose, 39.62% hemicellulose, and 13.63% lignin. Each reactor contained corncob particles, bacteria cells, and production medium. The effect of particle size (40–60 mesh, 60–80 mesh, and 80–100 mesh), strain of bacteria (A 002 and M 015), and the concentration of secondary carbon source on the glucose concentration was investigated. In addition, glucose production using the isolated bacteria was compared with a commercial enzyme. High performance liquid chromatography with a refractive index detector was used to determine the quantity of glucose. The optimum condition of microbial hydrolysis using bacteria isolated from Thai higher termites was found to be 80–100 mesh of corncob particle, with strain A 002 at 37 °C. The maximum glucose concentration was 0.60 g/L at 8 h.

บทคัดย่อ

ณัฐวุฒิ หอกิตติกุล: การผลิตกลูโคสจากซังข้าวโพคโดยกระบวนการย่อยด้วยจุลินทรีย์ ที่แยกได้จากปลวกชั้นสูง (Glucose Production from Corncob by Microbial Hydrolysis Using Bacteria Isolated from Thai Higher Termites) อ. ที่ปรึกษา: รศ. คร. ปราโมช รังสรรค์วิจิตร และ ศ. คร. สุเมธ ชวเดช 65 หน้า

งานวิจัยนี้เป็นการวิเคราะห์กระบวนการย่อยชังข้าวโพดด้วยจุลินทรีย์ (Microbial Hydrolysis of Corncob) ให้เป็นน้ำตาลกลูโคส โดยใช้แบคทีเรียที่แยกได้จากปลวกชั้นสูงซึ่งมี ความสามารถในการผลิตเซลลูเลส สำหรับการย่อยในแต่ละเครื่องปฏิกรณ์แบบกะประกอบไป ด้วย ซังข้าวโพด แบคทีเรีย และแหล่งอาหารของแบคทีเรีย ซึ่งซังข้าวโพดที่ใช้ประกอบด้วย เซลลูโลส 43.82 % เฮมิเซลลูโลส 39.62 % และลิกนิน 13.63 % การทดลองนี้ศึกษาอิทธิพลของ ขนาดของชังข้าวโพด (40–60, 60–80, และ 80–100 เมช) แบคทีเรียบาซิลลัส ซับทีลิสสายพันธุ์ เอ 002 และเอ็ม 015 และปริมาณมอลท์ในแหล่งอาหารของแบคทีเรีย (6, 8, 10, และ 12 กรัมต่อ ลิตร) นอกจากนี้ยังทำการเปรียบเทียบปริมาณน้ำตาลกลูโคสที่ผลิตได้ระหว่างการใช้แบคทีเรียกับ เอมไซม์เซิงพาณิชย์ น้ำตาลที่ได้วิเคราะห์ด้วยเครื่อง HPLC (high performance liquid chromatography) พบว่าสภาวะที่เหมาะสมในการผลิตน้ำตาลกลูโคส คือ การย่อยซังข้าวโพด ขนาด 80–100 เมช ด้วยแบคทีเรียสายพันธ์เอ 002 ที่ 37 องศาเซลเซียส ซึ่งได้ปริมาณน้ำตาล กลูโคสสูงสุด คือ 0.60 กรัมต่อลิตร นอกจากนี้พบว่าปริมาณมอลท์สกัดที่ใช้มีผลต่อปริมาณน้ำตาล ที่ได้ โดยปริมาณมอลท์ที่เหมาะสมคือ 12 กรัมต่อลิตร

ACKNOWLEDGEMENTS

This thesis work would have never been possible without the assistance of the following persons and organizations:

Firstly, I would like to express my deepest appreciation to Assoc. Prof. Pramoch Rangsunvigit and Prof. Sumaeth Chavadej for all of their excellent guidance, useful recommendations, creative comments, intensive attention, and encouragement throughout the course of research. They have not only taught me about the theoretical knowledge but also made me realize in myself that this research is very challenging. I feel proud to have been their student.

Furthermore, I would like to thank Ms. Supitcha Visuttitewin, Ms. Achiraya Jiraprasertwong and Ms. Kessara Seneesrisakul at the Petroleum and Petrochemical College, Chulalonglongkorn University, for advices and friendships throughout my research.

I would like to express my sincere thank to the Thaioil, the National Research University Project under the Ministry of Education, and the Center of Excellence on Petrochemical and Materials Technology, Thailand for providing the financial support for this thesis work.

My gratitude is absolutely extended to all staffs of the Petroleum and Petrochemical College, Chulalongkorn University, for all their kind assistance and cooperation.

Finally, I would like to take this opportunity to thank all of my PPC friends and Ph.D. students in room 612 for their friendly assistance, cheerfulness, and encouragement. Also, I am greatly indebted to my parents and my family for their support, love, and understanding.

TABLE OF CONTENTS

Title Page	i
Abstract (in English)	iii
Abstract (in Thai)	iv
Acknowledgements	v
Table of Contents	vi
List of Tables	ix
List of Figures	xii

CHAPTER

I	INTRODUCTION	1
II	LITERATURE REVIEW	3
	2.1 Lignocellulosic Biomass Materials	3
	2.2 Chemical Structure and Basic Component of	
	Lignocellusic Materials	5
	2.2.1 Cellulose	6
	2.2.2 Hemicellulose	7
	2.2.3 Lignin	7
	2.3 Glucose	8
	2.4 Sugar Production from Lignocellulosic Materials	9
	2.4.1 Concentrated Acid Hydrolysis	9
	2.4.2 Dilute Acid Hydrolysis	10
	2.4.3 Enzymatic Hydrolysis	10
	2.5 Cellulase Enzymes	12
	2.6 Enzyme from Termites	18
	2.7 Corncob	20

СНА	PTER
-----	------

III	EXPERIMENTAL	22
	3.1 Materials and Equipment	22
	3.2 Experimental Procedures	23
	3.2.1 Preparation of Corncob and Composition Analysis	23
	3.2.2 Preparation of Bacteria Cells for Microbial Hydrolysis	23
	3.2.3 Microbial Hydrolysis	24
	3.2.4 Enzymatic Hydrolysis	24
	3.2.5 Determination of Sugar and Bacteria Concentrations	24
IV	RESULTS AND DISCUSSION	25
	4.1 Corncob Composition	25
	4.2 Hydrolysis Capacity Value (HC)	26
	4.3 Microbial Hydrolysis	26
	4.3.1 Effect of Corncob Particle Size	27
	4.3.2 Effect of Bacteria strains	29
	4.2.3 Effect of Concentration of Secondary Carbon Source	31
	4.2.4 Glucose and Bacteria Evolutions	32
	4.4 Enzymatic Hydrolysis	33
	4.4.1 Effect of Cellulase Enzyme Loading	33
	4.5 Comparison of Glucose Production from Microbial	
	Hydrolysis and Enzymatic Hydrolysis	35
	4.6 Structure of Hydrolyzed Corncob Samples	36
V	CONCLUSIONS AND RECOMMENDATIONS	38
	5.1 Conclusions	38
	5.2 Recommendations	38
	REFERENCES	39

APPENDICES		42
Appendix A	Standard Calibration Curve	42
Appendix B	Media for Microorganisms	43
Appendix C	Reagent Preparations	44
Appendix D	Bacteria Concentration	45
Appendix E	Experiment Data of Hydrolysis	48
Appendix F	SEM images of before and after Hydrolysis	
	of corncob	61

CURRICULUM VITAE 65

LIST OF TABLES

TABI	$-\mathbf{E}$	PAGE
2.1	Major benefits of biofuels	4
2.2	Approximate composition (as a percentage) of various	
	biomass materials or agriculture waste products	5
2.3	Comparison of process conditions and performance of three	
	hydrolysis processes	11
2.4	Some of main enzymes required to degrade lignocelluloses	
	to monomers	12
2.5	Composition of cellulosic waste materials	15
2.6	Characteristics of isolates A 002, M 015, and F 018 by	
	microbiological methods	19
2.7	Composition of corncobs, corn stover, and switchgrass	21
4.1	Elemental composition of corncob	25
4.2	Chemical composition of corncob	25
4.3	Hydrolysis capacity values of bacterial strain A 002 and	
	strain M 015	26
4.4	Specific surface area of corncob particle	29
4.5	Specific surface area of 80-100 mesh corncob after	
	hydrolysis with bacterial strain A 002 and M 015	37
Al	Glucose calibration curve	42
El	Glucose produced from the hydrolysis of 40-60 mesh	
	corncob using the bacterial strain A 002 at 37 °C	48
E2	Glucose produced from the hydrolysis of 60-80 mesh	
	corncob using the bacterial strain A 002 at 37 °C	49
E3	Glucose produced from the hydrolysis of 80-100 mesh	
	corncob using the bacterial strain A 002 at 37 °C	50
E4	Glucose produced from the hydrolysis of 40-60 mesh	
	corncob using the bacterial strain M 015 at 37 °C	51

E5

E6

E7

E8

E9

E10

E11

E12

E13

Glucose produced from the hydrolysis of 60-80 mesh 52 corncob using the bacterial strain M 015 at 37 °C Glucose produced from the hydrolysis of 80-100 mesh 53 corncob using the bacterial strain M 015 at 37 °C Glucose produced from the hydrolysis of 80-100 mesh corncob using malt extract loading 12 g/L and bacterial strain A 002 at 37 °C 54 Glucose produced from the hydrolysis of 80-100 mesh corncob using malt extract loading 10 g/L and bacterial 55 strain A 002 at 37 °C Glucose produced from the hydrolysis of 80-100 mesh corncob using malt extract loading 8 g/L and bacterial strain 56 A 002 at 37 °C Glucose produced from the hydrolysis of 80-100 mesh corncob using malt extract loading 6 g/L and bacterial strain 57 A 002 at 37 °C Glucose produced from the enzymatic hydrolysis of 80–100 mesh corncob using 100 U cellulase enzyme loading at 37 °C 57 Glucose produced from the enzymatic hydrolysis of 80-100 mesh corncob using 200 U cellulase enzyme loading at 37 °C 58 Glucose produced from the enzymatic hydrolysis of 80–100 mesh corncob using 300 U cellulase enzyme loading at 37 °C 58 Glucose produced from the enzymatic hydrolysis of 80-100

PAGE

59

E15	Glucose produced from the enzymatic hydrolysis of 80-100	
	mesh corncob using 400 U cellulase enzyme loading at	
	37 °C	59
E16	Bacteria evolution from the hydrolysis of 80-100 mesh	
	corncob with the bacterial strain A 002 at 37 $^{\circ}\text{C}$	60
E17	Bacteria evolution from the hydrolysis of 80-100 mesh	
	corncob with the bacterial strain M 015 at 37 °C	60

LIST OF FIGURES

FIGURE

2.1 Representation of lignocellulosic structure showing, cellulose, hemicellulose, and lignin fractions 6 2.2 Schematic representation of a cellulose chain 6 2.3 Schematic of the basic structure of hemicellulose 7 8 2.4 Lignin building blocks Overall view of sugar and ethanol productions from 2.5 9 lignocellulosic materials Dilute acid hydrolysis 11 2.6 13 2.7 Mode of action of cellulolytic enzymes 2.8 Schematic representation of the cellulose enzymes over the cellulose structure 13 29 Picture of corncobs 20 Effect of corncob particle size on the produced glucose 4.1 concentration from the hydrolysis of corncob at 37 °C using 28 strain A 002 Effect of corncob particle size on the produced glucose 4.2 concentration from the hydrolysis of corncob at 37 °C using 28 strain M 015 4.3 Effect of bacterial strains on the glucose concentration

- produced from the hydrolysis of 80–100 mesh corncob at 37 °C.
 4.4 Effect of malt extract loading on the produced glucose concentration from the hydrolysis of 80–100 mesh corncob using strain A 002 at 37 °C.
- 4.5 Glucose and bacteria evolutions from the hydrolysis of the
 80-100 mesh corncob with the strain A 002 bacteria at
 37 °C.

xii

PAGE

32

4.6	Glucose and bacteria evolutions from the hydrolysis of the	
	80-100 mesh corncob with the strain M 015 bacteria at	
	37 °C	33
4.7	Effects of cellulase enzyme loading on the glucose	
	concentration produced from the hydrolysis of the 80-100	
	mesh corncob at 37 °C	34
4.8	The maximum glucose produced from the hydrolysis of the	
	80-100 mesh corncob at 37 °C by varied cellulase enzyme	
	loading	35
4.9	Scanning electron micrographs of the 80-100 mesh corncob	
	surfaces	36
Al	The relationship between glucose concentration (g/L) and	
	area	42
Dl	Diagram for determination the amount of nitrogen in	
	bacteria	45
D2	Procedure for analyzing amount of nitrogen	46
Fl	Scanning electron micrographs of the corncob surface before	
	hydrolysis	61
F2	Scanning electron micrographs of the 60-80 mesh corncob	
	surface after hydrolysis using strain A 002 at 37 °C	61
F3	Scanning electron micrographs of the 80-100 mesh corncob	
	surface after hydrolysis using strain A 002 at 37 °C	62
F4	Scanning electron micrographs of the 80-100 mesh corncob	
	surface after hydrolysis using strain M 015 at 37 $^{\circ}$ C	62
F5	Scanning electron micrographs of the 80-100 mesh corncob	
	surface after enzymatic hydrolysis using 100 U cellulase	
	enzyme loading at 37 °C	63

FIGURE

PAGE

F6	Scanning electron micrographs of the 80-100 mesh corncob	
	surface after enzymatic hydrolysis using 300 U cellulase	
	enzyme loading at 37 °C	63
F7	Scanning electron micrographs of the 80-100 mesh corncob	
	surface after enzymatic hydrolysis using 500 U cellulase	
	enzyme loading at 37 °C	64