

PRODUCTION OF BIOETHANOL FROM THAI GRASSES

Tidarat Komolwanich

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
The Petroleum and Petrochemical College, Chulalongkorn University
in Academic Partnership with
The University of Michigan, The University of Oklahoma,
and Case Western Reserve University
2016

Thesis Title: Production of Bioethanol from Thai Grasses
By: Tidarat Komolwanich
Program: Polymer Science
Thesis Advisors: Assoc. Prof. Sujitra Wongkasemjit
Assoc. Prof. Thanyalak Chaisuwan

Accepted by The Petroleum and Petrochemical College, Chulalongkorn University, in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

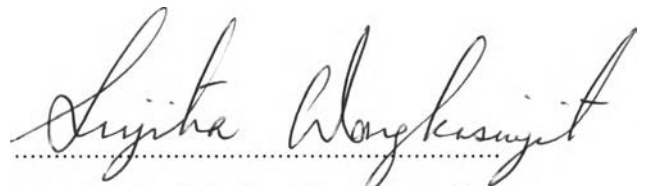


..... College Dean
(Asst. Prof. Pomthong Malakul)

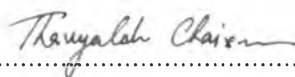
Thesis Committee:




.....
(Asst. Prof. Pomthong Malakul)



.....
(Assoc. Prof. Sujitra Wongkasemjit)



.....
(Assoc. Prof. Thanyalak Chaisuwan)



.....
(Assoc. Prof. Apanee Luengnaruemitchai)



.....
(Asst. Prof. Bussarin Ksapabutr)

ABSTRACT

5592001063: Polymer Science Program

Tidarat Komolwanich: Production of Bioethanol from Thai Grasses.

Thesis Advisors: Assoc. Prof. Sujitra Wongkasemjit, and Assoc. Prof. Thanyalak Chaisuwan 166 pp.

Keywords: Bioethanol production/ Lignocellulosic biomass/ Two-stage pretreatment/ Microwave irradiation/ *Saccharomyces cerevisiae*

Two-stage microwave pretreatment (microwave/NaOH pretreatment followed by microwave/H₂SO₄ pretreatment) successfully released monomeric sugars from various types of Thai grasses, namely, Mission grass (*Pennisetum polystachyon*), Kans grass (*Saccharum spontaneum*), Giant reed (*Arundo donax*), and Tiger grass (*Thysanolaena maxima*). The optimum conditions of the pretreatment were investigated, and the maximum monomeric sugar yields were compared. The microwave-assisted NaOH and H₂SO₄ pretreatments with 15:1 liquid-to-solid ratio were studied by varying chemical concentration, reaction temperature, and reaction time to optimize the amount of the monomeric sugars. The changes in structure of grasses were characterized using fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM). Of all grasses studied, the one giving the most amount of monomeric sugar was chosen to study the ethanol production. After the grass underwent through the two-stage pretreatment, it was subjected to enzymatic hydrolysis. Glucose, the source of ethanol fermentation, was obtained after the hydrolysis process. The grass hydrolyzate was overlimed at various pHs; and then sodium sulfite was added to remove inhibitory compounds and degradation products, such as furfural and hydroxymethylfurfural. Yeast population count was studied under a microscope. The change of glucose concentration in the hydrolyzate was detected by high performance liquid chromatography (HPLC), and the production of ethanol was determined using gas chromatography (GC).

บทคัดย่อ

ธิดารัตน์ โกมลวานิช: การผลิตเอทานอลเพื่อใช้เป็นเชื้อเพลิงจากหญ้าไทย (Production of Bioethanol from Thai Grasses) อ. ที่ปรึกษา: รองศาสตราจารย์ ดร. สุจิตรา วงศ์เกษมจิตต์ และรองศาสตราจารย์ ดร. ธีญญลักษณ์ ฉายสุวรรณ 166 หน้า

กระบวนการปรับสภาพพืชสองขั้นตอนด้วยรังสีไมโครเวฟและสารเคมีที่ใช้เป็นตัวเร่งปฏิกิริยาสามารถนำมาใช้ปรับสภาพพืชเพื่อสกัดน้ำตาลโมเลกุลเดี่ยวจากหญ้า โดยในงานวิจัยนี้ได้นำตัวอย่างหญ้าไทย ได้แก่ หญ้าขจรจบดอกเล็ก หญ้าดอกเลา คันท้อ และหญ้างัง มาใช้ในการศึกษา เนื่องจากมีสัดส่วนน้ำตาลโมเลกุลเดี่ยวสูงที่สุด โดยในงานวิจัยนี้ได้ศึกษาสภาวะของกระบวนการปรับสภาพพืชสองขั้นตอนด้วยรังสีไมโครเวฟและสารเคมีที่ใช้เป็นตัวเร่งปฏิกิริยาที่อัตราส่วนของเหลวต่อของแข็ง 15:1 และทำการศึกษาผลของอุณหภูมิ เวลา และความเข้มข้นของตัวเร่งปฏิกิริยา เพื่อหาสภาวะที่ดีที่สุดที่สามารถสกัดน้ำตาลโมเลกุลเดี่ยวจากหญ้า โดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดและเทคนิคฟูเรียรทรานสฟอร์มสเปกโตรสโคปี ศึกษาการเปลี่ยนแปลงโครงสร้างทางกายภาพและทางเคมีของตัวอย่างหญ้าจากกระบวนการปรับสภาพพืชและหญ้าที่สามารถสกัดน้ำตาลโมเลกุลเดี่ยวปริมาณมากที่สุดถูกนำมาศึกษาการผลิตเอทานอล ซึ่งหลังจากตัวอย่างหญ้าผ่านขั้นตอนการปรับสภาพพืช นำหมักจากหญ้าจะนำมาใช้ในขั้นตอนการย่อยสลายเซลลูโลสเพื่อให้ได้กลูโคสโดยการใช้เอนไซม์ และการหมักเพื่อผลิตเอทานอลด้วยยีสต์ โดยทำการศึกษาผลของค่าความเป็นกรดต่างที่ส่งผลต่อการกำจัดสารยับยั้งการผลิตเอทานอลด้วยยีสต์ ผลของค่าความเป็นกรดต่าง เวลา และสายพันธุ์ของยีสต์เพื่อหาสภาวะที่ดีที่สุดในการผลิตเอทานอล การเปลี่ยนแปลงของประชากรของยีสต์ ความเข้มข้นของน้ำตาลกลูโคสและเอทานอลถูกนำมาศึกษาโดยใช้กล้องจุลทรรศน์ ไฮเปอร์ฟอร์แมนซ์ลิควิดโครมาโตกราฟี และก๊าซโครมาโตกราฟี ตามลำดับ

ACKNOWLEDGEMENTS

Firstly, I would like to gratefully give thanks to my research advisor, Assoc. Prof. Sujitra Wongkasemjit, who is the major part of my success. She provides not only opportunities but also encouragement, valuable advice, and motivation. Moreover, I would like to especially thank her for believing in me. In my view, she is more than an advisor. She is like a mother who wants to see the success of her children.

I would like to sincerely thank another research advisor, Assoc. Prof. Thanyalak Chaisuwan who inspires, assists, and guides me when I face the problems. I wish to thank my thesis committee, Asst. Prof. Pomthong Malakul, Assoc. Prof. Apanee Luengnaruemitchai and Asst. Prof. Bussarin Ksapabutr, for their suggestion.

Another important part of my success is my colleague team: Sirirat Prasertwasu, Patomwat Tatijarern, and Darin Khumsupan. They are an excellent teamwork with sharing and supporting each other, advice, joyfulness, happiness, and great friendship. Nevertheless, they also gave me a valuable experience and good memories.

I would like to specially thank Dr. Rujirat Longloilert, Dr. Thitirat Inprasit, and Chayanaphat Chokradjaroen who always give me guidance, inspired idea, and great assistance. Furthermore, I would like to also thank Nuntapron Trisinsomboon for her help and kindness.

I am grateful for the partial scholarship and partial funding of the thesis work provided by the Petroleum and Petrochemical College; and the Excellence Center for Petrochemicals and Materials Technology, Chulalongkorn University.

I am genuinely thankful to Sureerat Jampa and Ratchadawan Yokubon who always cheer me up throughout my study for Ph.D. They provided kindness, helpful advice, great motivation, and powerful encouragement. Many thanks go to them for supporting me.

Lastly, I would like to praise my parents for their sacrifice, understanding, unconditioned love, gentle support, and powerful inspiration. They are the most significant part of this achievement.

TABLE OF CONTENTS

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

CHAPTER

I	INTRODUCTION	1
II	THEORETICAL BACKGROUND AND LITERATURE REVIEW	3
	2.1 Biomass-based Fuel	3
	2.2 Bioethanol	4
	2.3 Lignocellulosic Biomass	6
	2.4 The Composition of Lignocellulosic Biomass	7
	2.5 Ethanol Conversion Process	13
	2.6 Pretreatment of Lignocellulosic Biomass	15
	2.7 Hydrolysis	22
	2.8 Fermentation	29
	2.9 Fermentation Techniques	31
III	EXPERIMENTAL	33
	3.1 Objectives	33
	3.2 Materials and Chemical	33
	3.3 Characterization	37

CHAPTER	PAGE
IV	
CAPABILITY OF THAI MISSION GRASS (<i>PENNISETUM POLYSTACHYON</i>) AS A NEW WEEDY LIGNOCELLULOSIC FEEDSTOCK FOR PRODUCTION OF MONOMERIC SUGAR	38
4.1 Abstract	38
4.2 Introduction	39
4.3 Experimental	40
4.4 Results and Discussion	44
4.5 Conclusions	51
4.6 Acknowledgements	51
4.7 References	51
V	
COMPARATIVE POTENTIALITY OF KANS GRASS (<i>SACCHARUM SPONTANEUM</i>) AND GIANT REED (<i>ARUNDO DONAX</i>) AS LIGNOCELLULOSIC FEEDSTOCKS FOR THE RELEASE OF MONOMERIC SUGARS BY MICROWAVE/ CHEMICAL PRETREATMENT	60
5.1 Abstract	60
5.2 Introduction	61
5.3 Experimental	62
5.4 Results and Discussion	66
5.5 Conclusions	73
5.6 Acknowledgements	74
5.7 References	74

CHAPTER	PAGE
VI	
EVALUATION OF HIGHLY EFFICIENT MONOMERIC SUGAR YIELD FROM THAI TIGER GRASS (<i>THYSANOLAENA MAXIMA</i>)	92
6.1 Abstract	92
6.2 Introduction	93
6.3 Experimental	95
6.4 Results and Discussion	98
6.5 Conclusions	106
6.6 Acknowledgements	106
6.7 References	106
VII	
EFFICIENT PROCESS FOR ETHANOL PRODUCTION FROM THAI MISSION GRASS (<i>PENNISETUM POLYSTACHION</i>)	119
7.1 Abstract	119
7.2 Introduction	120
7.3 Experimental	121
7.4 Results and Discussion	124
7.5 Conclusions	132
7.6 Acknowledgements	133
7.7 References	133
VIII	
CONCLUSIONS AND RECOMMENDATIONS	142
REFERENCES	143
CURRICULUM VITAE	165

LIST OF TABLES

TABLE		PAGE
CHAPTER II		
2.1	The major raw material in each country	4
2.2	Composition of various types of lignocellulosic-biomass material	9
2.3	Advantages and disadvantages of various pretreatment processes for lignocellulosic materials	21
CHAPTER IV		
4.1	The chemical compositions of mission grass (<i>Pennisetumpolystachyon</i>)	55
4.2	Chemical composition of mission grass solid residues from each pretreatment stages	56
CHAPTER V		
5.1	Chemical composition of kans grass (<i>S.spontaneum</i>) and giant reed (<i>A.donax</i>)	79
5.2	Chemical composition of kans grass (<i>S.spontaneum</i>) solid residues from each pretreatment stage	80
5.3	Chemical composition of giant reed (<i>A.donax</i>) solid residues from each pretreatment stage	81
CHAPTER VI		
6.1	Chemical compositions of <i>T.maxima</i> solid residues from each stage	111

TABLE		PAGE
CHAPTER VII		
7.1	The chemical compositions of MG obtained from Tak and Nakhon Ratchasima Provinces, Thailand	138
7.2	Inhibitor concentration of mission grass (<i>P. polystachion</i> , Tak Province) from before and after overliming treatment at pH 10	138

LIST OF FIGURES

FIGURE		PAGE
CHAPTER II		
2.1	Reduction in GHG emissions by bioethanol produced from a variety of feedstocks on a life-cycle basis compared to gasoline.	5
2.2	Representation of lignocelluloses structure showing cellulose, hemicellulose and lignin fractions.	7
2.3	The chemical composition ratio of lignocelluloses.	8
2.4	Fragment (repeating unit) of a cellulose chain.	9
2.5	Illustration of a cellulose chain.	10
2.6	The monomer of hemicelluloses.	11
2.7	The ethanol production process.	13
2.8	Generic block diagram of bioethanol production from lignocelluloses biomass. Possibilities for reaction-reaction integration are shown inside the shaded boxes: SSF-simultaneous saccharification and fermentation; SSFC-simultaneous saccharification and co-fermentation. Main stream components are: C-cellulose; H-hemicellulose; L-lignin, G-glucose; P-pentose; I-inhibitors; EtOH-ethanol.	15
2.9	Schematic of goals of pretreatment on lignocellulosic material.	16
2.10	Energy requirements for ball milling municipal solid waste.	17
2.11	Main degradation products occurring during hydrolysis of lignocellulosic material.	22
2.12	Dilute acid hydrolysis (first-stage and two-stages) and separate fermentation of pentose and hexose sugars.	24
2.13	Schematic represent of the cellulase enzymes over the cellulose structure.	26

FIGURE	PAGE
2.14 Polymeric chemical structure of hemicelluloses and targets of hydrolytic enzymes involved in hemicellulosic polymer degradation.	27

CHAPTER IV

4.1 The glucose, xylose, arabinose components and the total monomeric sugar yield of untreated mission grass (<i>Pennisetumpolystachyon</i>) using 0.5 % (w/v) NaOH, 15:1 LSR, different times and temperatures: a) 40°, b) 60°, c) 80°, d) 100°, e) 120 °C, and f) comparison of the total monomeric sugar yields.	57
4.2 The glucose, xylose, arabinose components and the total monomeric sugar yield of microwave-assisted NaOH pretreated mission grass using 0.5 % (w/v) H ₂ SO ₄ , 15:1 LSR at different temperatures for different times: a) 80°, b) 100°, c) 140°, d) 160°, e) 180°, f) 200 °C, and (g) comparison of the total monomeric sugar yields.	58
4.3 a) Effect of NaOH concentration (% w/v) on monomeric sugar yields at 120 °C, 10 min, using 15:1 LSR and b) effect of H ₂ SO ₄ concentration (% w/v) on monomeric sugar yields of microwave-assisted NaOH pretreated mission grass at 200 °C, 5 min, using 15:1 LSR.	59
4.4 a) % Solid loss and c) solution pH of untreated Mission grass at different temperatures for different times using 0.5 % (w/v) NaOH and 15:1 LSR, b) % solid loss and d) solution pH of the microwave-assisted NaOH pretreated mission grass at different temperatures for different times using 0.5 % (w/v) H ₂ SO ₄ and 15:1 LSR.	59

FIGURE	PAGE
CHAPTER V	
5.1 The yields of glucose, xylose, and arabinose of untreated <i>S.spontaneum</i> using 0.5 % (w/v) NaOH, 15:1 liquid to solid ratio (LSR) at different times and temperatures: a) 40°, b) 80°, c) 120 °C, and d) comparison of total monomeric sugar yields.	82
5.2 The yields of glucose, xylose, and arabinose of untreated <i>A.donax</i> using 0.5 % (w/v) NaOH, 15:1 liquid to solid ratio (LSR) at different times and temperatures: a) 40°, b) 80°, c) 120 °C, and d) comparison of total monomeric sugar yields.	83
5.3 Effect of NaOH concentration (% w/v) on monomeric sugar yields of a) <i>S.spontaneum</i> at 80 °C, 5 min, using 15:1 LSR and b) <i>A.donax</i> at 120 °C, 5 min, using 15:1 LSR.	84
5.4 The yields of glucose, xylose, and arabinose of microwave-assisted NaOH pretreated <i>S.spontaneum</i> using 0.5 % (w/v) H ₂ SO ₄ , 15:1 LSR at different times and temperatures : a) 80°, b) 160°, c) 200 °C, and d) comparison of total monomeric sugar yields.	85
5.5 The yields of glucose, xylose, and arabinose of microwave-assisted NaOH pretreated <i>A.donax</i> using 0.5 % (w/v) H ₂ SO ₄ , 15:1 LSR at different times and temperatures: a) 100°, b) 140°, c) 180 °C, and (d) comparison of total monomeric sugar yields.	86
5.6 Effect of H ₂ SO ₄ concentration (% w/v) on monomeric sugar yields from microwave-assisted NaOH pretreated of a) <i>S.spontaneum</i> at 200 °C, 10 min, using 15:1 LSR and b) <i>A.donax</i> at 180 °C, 30 min, using 15:1 LSR.	87

FIGURE	PAGE
5.7 a) and c) % Solid loss of untreated <i>S.spontaneum</i> and <i>A.donax</i> , respectively, at various temperatures and times using 0.5 % (w/v) NaOH and 15:1 LSR. b) and d) % Solid loss of microwave-assisted NaOH pretreated of <i>S.spontaneum</i> and <i>A.donax</i> , respectively, at various temperatures and times using 0.5 % (w/v) H ₂ SO ₄ and 15:1 LSR.	88
5.8 a) and c) pH of untreated <i>S.spontaneum</i> and <i>A.donax</i> . respectively, at various temperatures and times using 0.5 % (w/v) NaOH and 15:1 LSR. b) and d) pH of microwave-assisted NaOH pretreated of <i>S.spontaneum</i> and <i>A.donax</i> , respectively, at various temperatures and times using 0.5 % (w/v) H ₂ SO ₄ and 15:1 LSR.	89
5.9 a) FTIR spectra of (A) raw, (B) microwave-assisted NaOH pretreated, and (C) two-stage pretreated <i>S.spontaneum</i> and b) FTIR spectra of (A) raw, (B) microwave-assisted NaOH pretreated, and (C) two-stage pretreated <i>A.donax</i> .	90
5.10 SEM images of (a) raw, (b) microwave-assisted NaOH pretreated, and (c) two-stage pretreated <i>S.spontaneum</i> at magnification 1000x, 1000x, and 10000x respectively.	91
5.11 SEM images of (a) raw, (b) microwave-assisted NaOH pretreated, and (c) two-stage pretreated <i>A.donax</i> at magnification 1000x, 2000x, and 2000x, respectively.	91
CHAPTER VI	
6.1 Schematic flowsheet for the conversion of <i>T. maxima</i> into bioethanol.	112

FIGURE	PAGE
6.2 The yields of glucose, xylose, and arabinose of untreated <i>T.maxima</i> at 120 °C and 15:1 liquid to solid ratio (LSR) at different NaOH concentration (% w/v) and time (5-60 min): a) 0.5 % (w/v), b) 1.0 % (w/v), c) 1.5 % (w/v), d) 2.0 % (w/v) and e) comparison of total monomeric sugar yields.	113
6.3 Effect of temperature on monomeric sugar yields of <i>T.maxima</i> using 0.5 % (w/v) NaOH, 15:1 LSR for 15 min.	114
6.4 The yields of glucose, xylose, and arabinose of pretreated <i>T.maxima</i> at 0.5 % (w/v) H ₂ SO ₄ and 15:1 LSR at different temperatures and times: a) 120°, b) 160°, c) 200° microwave-assisted NaOH, and d) comparison of total monomeric sugar yields.	114
6.5 The monomeric sugar yields of <i>T.maxima</i> at 200 °C/5 min and 15:1 LSR: a) effect of acid concentration and b) effect of acid type using 0.5 % (w/v) acid concentration.	115
6.6 The yields of glucose, xylose, and arabinose of enzymatic hydrolyzed <i>T.maxima</i> at pH 4.8, 160 µl of <i>T.reesei</i> ATCC 26921 loading at different temperatures and times: a) 45°, b) 50°, c) 55°, and d) comparison of total monomeric sugar yields.	116
6.7 The monomeric sugar yields a) effect of varying pH (3.0-5.6) by <i>Trichoderma reesei</i> ATCC 26921, b) effect of enzyme loading (40-280 µl) by <i>Trichoderma reesei</i> ATCC 26921, and c) effect of enzyme type, using 160 µl of enzyme loading at 45 °C/ 120 h.	117
6.8 FTIR spectra of (A) raw, (B) microwave-assisted NaOH pretreated, (C) two-stage pretreated, and (D) enzymatic hydrolyzed <i>T.maxima</i> .	118

FIGURE		PAGE
CHAPTER VII		
7.1	The effect of overliming in comparison to the hydrolyzate before overliming.	139
7.2	a) Yeast (<i>S. cerevisiae</i> TISTR 5049) population at various overliming pHs per incubation time, b) glucose consumption of <i>S. cerevisiae</i> TISTR 5049 per incubation time, and c) ethanol production of MG hydrolyzate by <i>S. cerevisiae</i> TISTR 5049 at overliming pH 8-12.	139
7.3	a) Various strains of baker's yeast (<i>S. cerevisiae</i>) count per incubation time at pH 10 overliming, b) glucose concentration per incubation time for various strains of baker's yeast <i>S. cerevisiae</i> at pH 10 overliming, and c) the production of ethanol from various strains of <i>S. cerevisiae</i> in 96 h.	141