CONTENTS

	PAGE
ENGLISH ABSTRACT	ii
THAI ABSTRACT	iv
ACKNOWLEDGEMENTS	vi
CONTENTS	vii
LIST OF TABLES	ix
LIST OF FIGURES	xii
CHAPTER	
1. INTRODUCTION	
1.1 Background	1
1.2 Objective	2
1.3 Scope of work	2
1.4 Expected result	3
2. THEORY AND LITERATURE REVIEW	
2.1 Theory	4
2.1.1 Bioethanol Production	4
2.2.2 Lignocellulose Material	5
2.2.3 Chemical hydrolysis	7
2.2.4 Pretreatment for Lignocellulose Pretreatment	9
2.2.5 Pretreatment Method	11
2.2.6 Chemical Pretreatment	14
2.2.7 Enzymatic Hydrolysis	14
2.2.8 Analysis Method	16
2.2.9 Response Surface Methodology (RSM)	19
2.2.10 Analysis of Variance (ANOVA)	24
2.2 Literature Review	29
3. METHODOLOGY	
3.1 Raw materials, equipment and chemical reagents	33
3.1.1 Reagents and materials	33
3.1.2 Equipment	34
3.2 Experiment Procedures	35
3.2.1 Raw Material	35
3.2.2 Chemical Pretreatment	35
3.2.3 Screening of Multivariable Parameters Affecting Lignocellulosic	36
Material Pretreatment Using Fractional Factorial Design (FFD)	
3.2.4 Pretreatment Hydrolysis	38
3.2.5 Statistical Analysis	38

		PAGE
4. RE	SULT AND DISCUSSION	
4.1	Acid Pretreatment Result	39
4.1.1	Sulfuric Acid (H ₂ SO ₄) Pretreatment	40
4.1.2	2 Hydrochloric Acid (HCl) Pretreatment	52
4.2	Alkaline Pretreatment Result	63
4.2.1	Sodium Hydroxide (NaOH) Pretreatment	63
4.2.2	2 Potassium Hydroxide (KOH) Pretreatment	71
4.3	Distillate Water Pretreatment	79
4.4	The Comparison of The Effective of The Chemical Pretreatment for Xylose Production	83
4.5	The Comparison of The Effective of The Chemical Pretreatment for Glucose Production	84
4.6	The Comparison of The Effective of The Chemical Pretreatment for Inhibitors Production	85
4.7	The Comparison of The Effective of The Chemical Pretreatment for Lignin Removal	90
4.8	The Validation of RSM Result	92
4.9	Enzymatic Hydrolysis	94
5. CO	NCLUSIONS AND RECCOMMENDATIONS	
5.1 (Conclusions	101
5.2 1	Recommendations	103
REFE	RENCES	103
APPE	NDIX	
A	Chemical concentration calculation	108
В	Pretreatment result	113
C	Analytical method	130
D		134
CURI	RICULUM VITAE	139

LIST OF TABLES

TA	BLE	PAGE
2.1	Pretreatment process of lignocellulose material	12
2.2	The design of experiment of 27 – 3 design	22
2.3	The Summary of One Factors ANOVA	25
2.4	The Summary of One Factors ANOVA	28
3.1	Coded and real values of FFD	36
3.2	Factional Factorial Design of Sodium hydroxide (NaOH) pretreatment	36
3.3	Factional Factorial Design of potassium hydroxide (KOH) pretreatment	37
3.4	Factional Factorial Design of Sulfuric acid (H ₂ SO ₄) pretreatment	37
3.5	Factional Factorial Design of Hydrochloric acid (HCl) pretreatment	37
4.1	Influence of temperature, time and concentration on monomeric sugar,	40
	furfural, HMF and soluble lignin in unit of g/l of H ₂ SO ₄ pretreatment	
4.2	Influence of temperature, time and concentration on monomeric sugar,	40
	furfural, HMF and soluble lignin in unit of g/gbiomass of H ₂ SO ₄ pretreatment	t
4.3	Effect estimates on xylose concentration from FFD of H ₂ SO ₄ pretreatment	45
4.4	Effect estimates on glucose concentration from FFD of H ₂ SO ₄ pretreatment	46
4.5	Effect estimates on furfural concentration from FFD of H ₂ SO ₄ pretreatment	47
4.6	Effect estimates on HMF concentration from FFD of H ₂ SO ₄ pretreatment	48
4.7	Effect estimates on lignin concentration from FFD of H ₂ SO ₄ pretreatment	49
4.8	Effect estimates on acetic acid concentration from FFD	50
	of H ₂ SO ₄ pretreatment	
4.9	Influence of temperature, time and concentration on monomeric sugar,	52
	furfural, HMF and soluble lignin in unit of g/l of HCl pretreatment	
4.10	Influence of temperature, time and concentration on monomeric sugar,	52
	furfural, HMF and soluble lignin in unit of g/gbiomass of HCl pretreatment	
4.11	Effect estimates on xylose concentration from FFD of HCl pretreatment	56
4.12	Effect estimates on glucose concentration from FFD of HCl pretreatment	57
	Effect estimates on furfural concentration from FFD of HCl pretreatment	58
	Effect estimates on HMF concentration from FFD of HCl pretreatment	59
4.15	Effect estimates on soluble lignin concentration from FFD	60
	of HCl pretreatment	
4.16	Effect estimates on acetic acid concentration from FFD	61
	of HCl pretreatment	
4.17	Influence of temperature, time and concentration on monomeric sugar,	63
	furfural, HMF and soluble lignin in unit of g/l of NaOH pretreatment	
4.18	Influence of temperature, time and concentration on monomeric sugar,	63
	furfural, HMF and soluble lignin in unit of g/gbiomass of NaOH pretreatmen	
4.19	Effect estimates on soluble lignin concentration from FFD of	68
	NaOH pretreatment	

LIST OF TABLES

TA]	BLE	PAGI
4.20	Effect estimates on acetic acid concentration from FFD of NaOH pretreatment	68
4.21	Influence of temperature, time and concentration on monomeric sugar, furfural, HMF and soluble lignin in unit of g/l of KOH pretreatment	71
4.22	Influence of temperature, time and concentration on monomeric sugar, furfural, HMF and soluble lignin in unit of g/gbiomass of KOH pretreatment	71
4.23	Effect estimates soluble lignin concentration from FFD of KOH pretreatment	76
4.24	Effect estimates on acetic acid concentration from FFD of KOH pretreatment	77
4.25	Influence of temperature, time and concentration on monomeric sugar, furfural, HMF and soluble lignin in unit of g/l of blank pretreatment	79
	Influence of temperature, time and concentration on monomeric sugar, furfural, HMF and soluble lignin in unit of g/gbiomass of blank pretreatment	79
4.27	Influence of temperature, time and concentration on monomeric sugar, furfural, HMF and soluble lignin in unit of g/l	92
4.28	Influence of temperature, time and concentration on monomeric sugar, furfural, HMF and soluble lignin in unit of g/gbiomass	92
4.29	The % Recovery of cellulose, hemicellulose and lignin product in the fitrate after pretreatment	99
B.1	The concentration result of monomeric sugar, acetic acid, citric acid, oxalic acid, furfural, HMF and soluble lignin in liquid phase after filtration in the unit of g/l from H ₂ SO ₄ pretreatment	114
B.2	The concentration result of monomeric sugar, acetic acid, citric acid, oxalic acid, furfural, HMF and soluble lignin in liquid phase after fitration in the unit of $g/g(biomass)$ from H_2SO_4 preatreament	116
B.3	The concentration result of monomeric sugar, acetic acid, citric acid, oxalic acid, furfural, HMF and soluble lignin in liquid phase after fitration in the unit of g/l from HCl preatreament	117
B.4	The concentration result of monomeric sugar, acetic acid, citric acid, oxalic acid, furfural, HMF and soluble lignin in liquid phase after fitration in the unit of g/g(biomass) from HCl preatreament	119
B.5 ′	The concentration result of monomeric sugar, acetic acid, citric acid, oxalic acid, furfural, HMF and soluble lignin in liquid phase after fitration in the unit of g/l from NaOH preatreament	120
	The concentration result of monomeric sugar, acetic acid, citric acid, oxalic acid, furfural, HMF and soluble lignin in liquid phase after fitration in the unit of g/g(biomass) from NaOH preatreament	122

LIST OF TABLES

TABLE	PAGE
B.7 The concentration result of monomeric sugar, acetic acid, citric oxalic acid, furfural, HMF and soluble lignin in liquid phase af fitration in the unit of g/l from KOH preatreament	
B.8 The concentration result of monomeric sugar, acetic acid, citric oxalic acid, furfural, HMF and soluble lignin in liquid phase affitration in the unit of $g/g_{(biomass)}$ from KOH preatreament	
B.9 The concentration result of monomeric sugar, acetic acid, citric oxalic acid, furfural, HMF and soluble lignin in liquid phase at fitration in the unit of g/l from blank preatreament	
B.10 The concentration result of monomeric sugar, acetic acid, citric oxalic acid, furfural, HMF and soluble lignin in liquid phase a fitration in the unit of $g/g_{(biomass)}$ from blank preatreament	
B.11 The concentration result of monomeric sugar, acetic acid, citric oxalic acid, furfural and HMF after hydrolysis at the reaction of 48 hr	
B.12 The concentration result of monomeric sugar, acetic acid, citric oxalic acid, furfural and HMF after hydrolysis at the reaction of 0 hr	

FIG	GURE	PAGE
2.1	Process of Bioethanol Production from Sugarcane Bagass	4
2.2	The lignocellulose material structure	5
2.3	The Cellulose Molecular Structure	5
2.4	The Hemicellulose Molecular Structure	6
2.5	The three common monolignols: paracoumaryl alcohol (1), coniferyl alcohol	6
	(2), and sinapyl alcohol (3)	
2.6	The mechanism of hydrolytic cleavage of glycosodic bonds	7
2.7	Mechanism of the β -O-4 bond cleavage on Lundgren research	8
2.8	Non-cyclic benzyl ether linkage in lignin	9
2.9	Reaction of β -ether model compound to form guaiacol and veratrylglycerol	9
2.10	The Cellulose Structure	10
2.11	Pretreatment process of lignocellulose material	11
2.12	Mechanism of enzyme of cellulase	15
2.13	High Liquid Performance Chromatography installments	16
2.14	A chromatogram of sample that analyzed by HPLC	17
2.15	UV-Visible Spectroscopy Installments	18
2.16	The derivation Beer-Lambert's Law	18
2.17	The 3 – dimensional response surface and the contour plot	20
2.18	2 ³ full factorial design (8 points)	21
2.19	A central composition design for 3 variables at 2 levels	23
2.20	Normal Probability Plot	26
2.21	Equal Variance Plot	26
2.22	Independent Error Plot	27
3.1	The flow diagram of work plan	33
4.1	Reactions occurring to carbohydrates during hydrolysis	39
4.2	The product concentration (g/l) with different pretreatment condition	41
	by H2SO4 pretreatment	
4.3	The product content (g/gbiomass) with different pretreatment condition	41
	by H2SO4 pretreatmen	
4.4	The product recovery (g/l) with different pretreatment condition by H ₂ SO ₄	43
	pretreatment	
4.5	Normal plot of residuals of xylose content of H ₂ SO ₄ pretreatment	45
4.6	Normal plot of residuals of glucose content of H ₂ SO ₄ pretreatment	46
4.7	Normal plot of residuals of furfural content of H ₂ SO ₄ pretreatment	47
4.8	Normal plot of residuals of HMF content of H ₂ SO ₄ pretreatment	48
4.9	Normal plot of residuals of soluble lignin content of H ₂ SO ₄ pretreatment	49
4.10	Normal plot of residuals of acetic acid content of H ₂ SO ₄ pretreatment	50
4.11	The product concentration (g/l) with different pretreatment condition by HC	1 53
	pretreatment	

FIGURE	PAGE
4.12 The product content (g/gbiomass) with different pretreatment condition pretreatment	HCl 53
4.13 The product recovery (g/l) with different pretreatment condition HCl pretreatment	55
4.14 Normal plot of residuals of xylose content of HCl pretreatment	56
4.15 Normal plot of residuals of glucose content of HCl pretreatment	57
4.16 Normal plot of residuals of furfural content of HCl pretreatment	58
4.17 Normal plot of residuals of HMF content of HCl pretreatment	59
4.18 Normal plot of residuals of soluble lignin content of HCl pretreatment	60
4.19 Normal plot of residuals of acetic acid content of HCl pretreatment	61
4.20 The product recovery (g/l) with different pretreatment condition NaOH pretreatment	64
4.21 The product content $(g/g_{biomass})$ with different pretreatment condition Nat pretreatment	OH 64
4.22 The product recovery (g/l) with different pretreatment condition NaOH pretreatment	66
4.23 Normal plot of residuals of soluble lignin content of NaOH pretreatment	68
4.24 Normal plot of residuals of acetic acid content of NaOH pretreatment	69
4.25 The product concentration (g/l) with different pretreatment condition KC pretreatment	OH 72
4.26 The product content $(g/g_{biomass})$ with different pretreatment condition KC pretreatment	OH 72
4.27 The product recovery (g/l) with different pretreatment condition KOH pretreatment	74
4.28 Normal plot of residuals of soluble lignin content of KOH pretreatment	76
4.29 Normal plot of residuals of acetic acid content of KOH pretreatment	77
4.30 The product concentration (g/l) with different pretreatment condition by distillated water pretreatment	80
4.31 The product content $(g/g_{biomass})$ with different pretreatment condition by distillated water pretreatment	80
4.32 The product recovery (g/l) with different pretreatment condition by distillated water pretreatment	82
4.33 The response surface plot on xylose production. A- Effect of reaction tin at H_2SO_4 concentration of 3% v/v and temperature of 115 °C. B - Effect of temperature and reaction time at HCl concentration of 3% v/v	
4.34 The acetal formation reaction	83
4.35 The response surface plot on glucose production. A-Effect of temperatur	
and H ₂ SO ₄ concentration at reaction time of 60 min. B-Effect of temperature and HCl concentration at reaction time of 60 min.	

FIGURE	PAGE
 4.36 The response surface plot on furfural production. A-Effect of temperature and H₂SO₄ concentration at reaction time of 60 min. B- Effect of temperature and HCl concentration at reaction time of 60 min 	86
4.37 The response surface plot on HMF production. A-Effect of temperature and H_2SO_4 concentration at reaction time of 60 min. B – Effect of temperature and HCl concentration at reaction time of 60 min	87
 4.38 The response surface plot on acetic acid production. A-Effect of temperature and H₂SO₄ concentration at reaction time of 60 min. B- Effect of HCl concentration at reaction time of 60 min and temperature of 115 °C. C- Effect of KOH concentration and temperature at reaction time of 60 min 	88 ature
4.39 The response surface plot on lignin removal. A- Effect of temperature at 3% v/v of H ₂ SO ₄ and reaction time of 60 min. B- Effect of temperature at 3% v/v of HCl and reaction time of 60 min. C- Effect of NaOH concentration at reaction time of 60 min and temperature of 115 °C. D- Effect of KOH concentration at temperature of 115 °C reaction time of 60 min.	ct
4.40 The product concentration (g/l) with different pretreatment condition	n 93
4.41 The product content (g/gbiomass) with different pretreatment condit	
4.42 The product concentration (g/l) with different pretreatment condition enzymatic hydrolysis at starting point (reaction time = 0)	n from 96
4.43 The product concentration (g/l) with different pretreatment conditio enzymatic hydrolysis for 48 hours	n from 97
 D.1 The response surface plot on xylose production. A-Effect of temperature and reaction time at HCl concentration of 3% v/v. B- Effect of reaction time and HCl concentration at temperature or 	135 f 115 °C.
D.2 The response surface plot on glucose production. A-Effect of temperature and reaction time at H_2SO_4 concentration of 3% v/v. B- Effect of reaction time and H_2SO_4 concentration at temperature of 115 °C.	135
D.3 The response surface plot on glucose production. A-Effect of temperature and reaction time at HCl concentration of 3% v/v. B-Effect of reaction time and HCl concentration at temperature of 115 °C.	136
D.4 The response surface plot on furfural production. A-Effect of temperature and reaction time at H_2SO_4 concentration of 3% v/v. I Effect of reaction time and H_2SO_4 concentration at temperature of 115 °C	

FIC	GURE	PAGE
D.5	The response surface plot on furfural production. A-Effect of temperature and reaction time at HCl concentration of 3% v/v. B-Effect of reaction time and HCl concentration at temperature of 115 °	137
D.6	The response surface plot on HMF production. A-Effect of temperature and reaction time at H_2SO_4 concentration of 3% v/v. B- Effect of reaction time and H_2SO_4 concentration at temperature of 115 °C	137
D.7	The response surface plot on HMF production. A-Effect of temperature and reaction time at HCl concentration of 3% v/v. B- Effect of reaction time and HCl concentration at temperature of 115 °C.	138
D.8	The response surface plot on soluble lignin production. A-Effect of concentration and reaction time at temperature of 115 °C.	138