

TABLE OF CONTENTS

TITLE SHEET	i
APPROVAL SHEET	ii
LEGISLATION LETTER	iii
DECLARATION OF AUTHENTICITY	iv
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	viii
LIST OF TABLES	xii
LIST OF FIGURES	xiii
ABSTRACT	xiv
INTISARI	xv
CHAPTER I INTRODUCTION	1
1.1 Background	1
1.2 Objectives	5
1.3 Research scheme	5
CHAPTER II LITERATURE STUDY	8
2.1 Bioethanol	8
2.2 Bioethanol Production	11
2.2.1 Pretreatment	11
2.2.2 Hydrolysis	12

2.2.3	Fermentation	16
2.2.4	Ethanol Recovery	17
2.3	Simultaneous Saccharification and Fermentation	17
2.4	Yeast	20
2.4.1	Ethanol producing yeast	21
2.4.2	Thermotolerant and osmotolerant of ethanol producing yeast	21
2.4.3	Yeast isolation	23
2.5	Sources of potential ethanol producers	24
2.5.1	Tape ketan	24
2.5.2	Ragi tape	25
2.5.3	Ethanol fermentation active media	26
2.5.4	Tropical Fruits	27
2.6	Cassava Pulp	31
2.7	Cassava Pulp Degrading Enzymes	33
2.7.1	α -Amylase	33
2.7.2	Glucoamylase	34
2.7.3	Cellulase	35
CHAPTER III MATERIALS AND METHOD		37
3.1	Sources of potential ethanol producers	37
3.2	Cassava pulp	37
3.3	Culture media	38

3.4	Enzymes	39
3.5	Isolation and identification of the microorganisms	39
3.6	Ethanol fermentation ability of the isolates	40
3.7	Reactivation of the yeast isolates and inoculum preparation	40
3.8	Growth of the isolates at various temperatures	40
3.9	Growth of the selected isolates at elevated temperature (42 °C)	41
3.10	Enzymatic saccharification of cassava pulp	41
3.11	Simultaneous saccharification and fermentation	42
3.12	Analyses	43
3.12.1	Microbial growth measurements	43
3.12.2	Reducing sugar determination	44
3.12.3	Ethanol determination by gas chromatography method	45
3.12.4	Determination of ethanol, glucose, maltose, and xylose concentrations	45
3.12.5	Calculation of hydrolysis efficiency	46
3.12.6	Calculation of ethanol fermentation efficiency	47
3.12.7	Statistical analysis	47
3.12.8	Estimation of kinetic parameters by modified Gompertz model	47
3.13	Time and location of the research	48
CHAPTER IV RESULTS AND DISCUSSION		49
4.1	Isolation and identification of potential ethanol producers	49

4.2	Ethanol fermentation ability of the isolates	52
4.3	Growth of the isolates at various temperatures	53
4.4	Growth of the selected isolates at 42 °C	54
4.5	Saccharification of cassava pulp	56
4.6	Simultaneous saccharification and fermentation	59
CHAPTER V CONCLUSION		65
REFERENCES		66
APPENDIX		75
Appendix 1. Morphology of colonies and cells of the isolates		75
Appendix 2. Ethanol fermentation ability of the isolates		78
Appendix 3. Growth of the isolates at various temperatures		79
Appendix 4. Growth of the isolates at 42 °C		80
Appendix 5. Preparation of DNS reagent		83
Appendix 6. Glucose standard curve		84
Appendix 7. Saccharification of cassava pulp		86
Appendix 8. HPLC standard curve		87
Appendix 9. Confirmation experiment of saccharification at 42 °C		89
Appendix 10. Simultaneous Saccharification and Fermentation		90
Appendix 11. Statistical analysis by paired T-test		92
Appendix 11. Documentations		94

LIST OF TABLES

Table 1 Physicochemical compositions of cassava pulp from several studies	33
Table 2 Potential isolates obtained from tape ketan, ethanol fermentation active media, jackfruit, durian, and ragi tape.....	50
Table 3 Characteristics of colonies and cells of the isolates.....	52
Table 4. Ethanol production of the Isolates	53
Table 5 Key results of SSF with and without pre-hydrolysis.	63
Table 6 Kinetic parameters for ethanol production from cassava pulp.	64

LIST OF FIGURES

Figure 1 Research scheme of this project.	7
Figure 2 Tape Ketan.....	25
Figure 3. Ragi Tape.....	26
Figure 4. Ethanol fermentation active media	27
Figure 5. Jackfruit	29
Figure 6. Durian	30
Figure 7 Basic process of cassava starch production in a starch factory.	32
Figure 8 Temperature and pH profiles of iKnowZyme® HTAA	34
Figure 9 Temperature and pH profiles of iKnowZyme® GA 200 L	35
Figure 10 Temperature and pH profiles of Cellic® CTec3 HS	36
Figure 11. Colonies characteristics on agar plates	51
Figure 12 Growth of the isolates at different temperatures.....	54
Figure 13 Growth of Isolates TKYF, BKYF, and RTYK in PGY medium at 42 °C.	55
Figure 14 Reducing sugar production from cassava pulp during saccharification	57
Figure 15 Production of glucose during saccharification of cassava pulp at 42 °C.	59
Figure 16 Glucose consumption and ethanol production by RTYK during SSF without pre-hydrolysis (A) and with pre-hydrolysis (B)..	61