

# CONTENTS

<b>No.</b>	<b>Title</b>	<b>Page</b>
<b>1.</b>	<b>Introduction</b>	<b>1</b>
<b>2.</b>	<b>Review of literature</b>	<b>7</b>
2.1	Microorganism	7
2.2	Substrate	7
2.3	Catabolite Repression	8
2.4	Random Amplified Polymorphic DNA	10
2.5	Invertase	12
2.6	Molasses	15
2.7	Nitrogen source	17
2.8	Phosphorous source	18
2.9	Inoculum	19
2.10	Ethanol Tolerance	19
2.11	Aeration	22
2.12	Thermotolerance	23
2.13	Very High Gravity Technology	27
2.14	By Products	29
2.15	Antibiotic	30
2.16	Response Surface Methodology	32
<b>3.</b>	<b>Materials and Methods</b>	<b>34</b>
3.1	Research Stations	34
3.2	Chemicals/Biochemicals	34
3.3	Substrate	34
3.4	Microorganism	34
3.5	Maintenance of the culture	34
3.5.1	Growth Medium Composition	35
3.5.2	Preparation of Plates	35
3.5.3	Preparation of Slants	35
3.6	Preparation of purified parent strain	36
3.6.1	Medium for inoculums	36
3.7	Strain Improvement	37
3.7.1	Survival Curve	37

3.7.2	Purification of Mutant culture	37
3.7.3	Selection of Mutant of <i>S. cerevisiae</i>	37
3.8	Genetic variability	38
3.8.1	Total Genomic DNA Isolation	38
3.8.2	RAPD Assay	38
3.8.2.1	Data analysis	39
3.9	Invertase and ethanol production at lab. scale	39
3.9.1	Effect of Nitrogen and Phosphate source	39
3.9.2	Effect of Temperature	40
3.9.3	Effect of pH	40
3.10	Industrial Scale Studies	40
3.10.1	Propagation of Yeast Culture	40
3.10.1.1	First Stage	40
3.10.1.2	2 <sup>nd</sup> stage	41
3.10.1.3	3 <sup>rd</sup> Stage	41
3.10.2	Fermentation	41
3.10.2.1	Effect of different brix (sugar level)	41
3.10.2.2	Effect of different inoculums size	41
3.10.2.3	Effect of different level rise	42
3.10.2.4	Effect of temperature	42
3.10.3	Effect of Antibiotic	42
3.10.3.1	Effect of Sodium Flouride	42
3.10.3.2	Effect of virginiamycin	42
3.10.3	Very High Gravity Technology	42
3.10.3.1	Effect of Very High Brix	42
3.10.3.2	Effect of Aeration Rate	42
3.11	Analysis	42
3.11.1	Invertase assay	42
3.11.1.1	Calculation of Invertase Activity	43
3.11.2	Brix	43
3.11.3	pH	43
3.11.4	Sugars Analysis	43
3.11.5	Cell Population	43

	3.11.6 Ethanol %	43
	3.11.7 Yied	43
	3.11.8 Fermentation efficiency	44
	3.11.9 Gas Chromatography	44
	3.11.10Potassium Permanganate Time Test (PTT)	44
	3.11.11Acidity of Alcohol	44
	3.12 Statistical Analysis	44
<b>4.</b>	<b>Results and Discussion</b>	<b>45</b>
	4.1 Genetic Variability between the mutant and parent strains	46
	4.1.1 Genomic DNA Isolation	46
	4.1.2 RAPD Assay	47
	4.2 Laboratory scale study	49
	4.3 Industrial Scale studies	102
	4.4 Use of antibiotic for ethanol fermentation from contaminated/deteriorated molasses	145
	4.4.1 Use of Virginiamycin	145
	4.4.2 Use of Sodium Flouride	148
	4.5 Very High Gravity Fermentation	151
	4.6 Conclusion	168
<b>5.0</b>	<b>Summary</b>	<b>169</b>
	<b>Literature cited</b>	<b>171</b>