ECONOMIC EVALUATION OF BIO-ETHANOL PRODUCTION FROM CASSAVA STARCH USING FUSANTS OF *WICKHERMOMYCES ANOMALUS*

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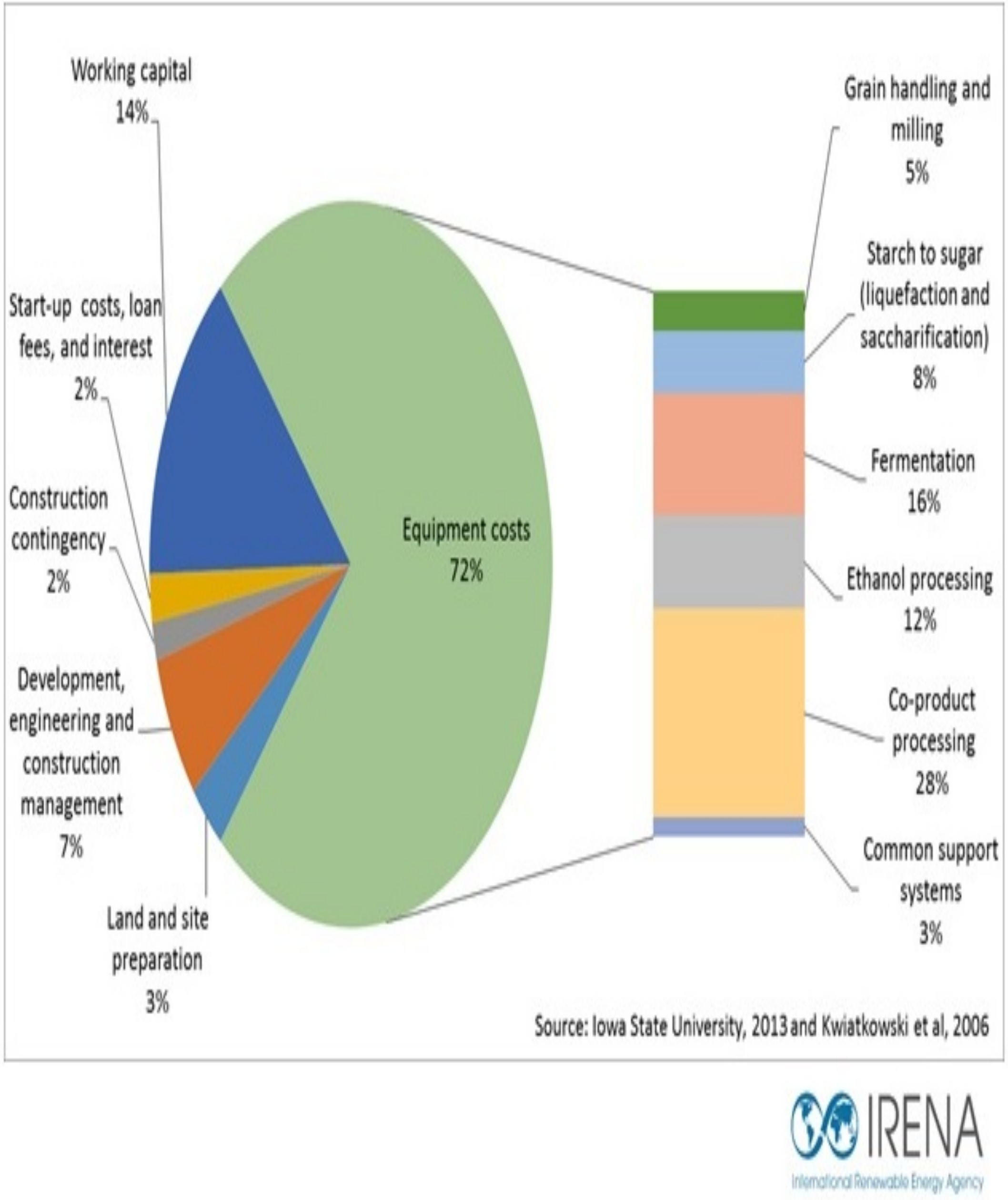
INTRODUCTION

- Ethanol among other another alternative fuels, is generating an increasing global interest (Nghiem *et al.,* 2010). The economics of its production is significantly influenced by the cost of raw materials.
- Of all the major ethanol crops, starchy materials give the highest yield (up to 6tonnes/ ha) (FAO stat, 2012; Leen *et. al., 2007*).
- The use of cassava, which is a relatively low cost material for production of commercially viable products such as ethanol will boost rural economies (FAO, 2012).
- One argument against the use of food crops for fuel ethanol production is that it can adversely affect food security. However, food production and distribution are highly depended on energy supply (Ogbonna and okoli, 2013).
- World bank (2013) report showed that there are more than 202 million hectares of usable uncultivated land in Africa and only about 6% of arable land is under effective cultivation in some countries (Gnansounou *et al*. (2007).



Strain Improvement by Protoplast Fusion Technique

- *Wickerhamomyces anomalus* is a non- *saccharomyces* yeast that has been associated with spoilage but in recent times interest in their beneficial roles have grown (Mateo and Maicas, 2016).

- The biotechnological applications of non- *saccharomyces* yeasts such as production of enzymes and ethanol are however, species- and strain-specific (Padilla *et al.,* 2016)

- The need to meet global demand for alcohol with a view of maximizing profits, has necessitated strain improvement programmes such as protoplast fusion which has been a successful tool in achieving genetic manipulation (Tokdar *et. al*., 2014).

OBJECTIVES

- To develop improved fermenters using protoplast fusion technique.

- To produce ethanol by fermentation of hydrolysed starch from cassava roots using palm wine yeasts.

- To estimate the profitability of the bio-ethanol production process form cassava starch on a small scale.

MATERIALS AND METHODS

ISOLATION AND CHARACTERIZATION OF *W. ANOMALUS* FROM PALM WINE

- **Isolation and identification of *W. anomalus* from palm wine**

Pure cultures of *W. anomalus* were isolated from aged palm wine samples obtained which were allowed to attain spoilage at room temperature. Standard procedures for identification was done by methods described by Phaff and Starmer (1987) and Barneth *et. al*. (1990).

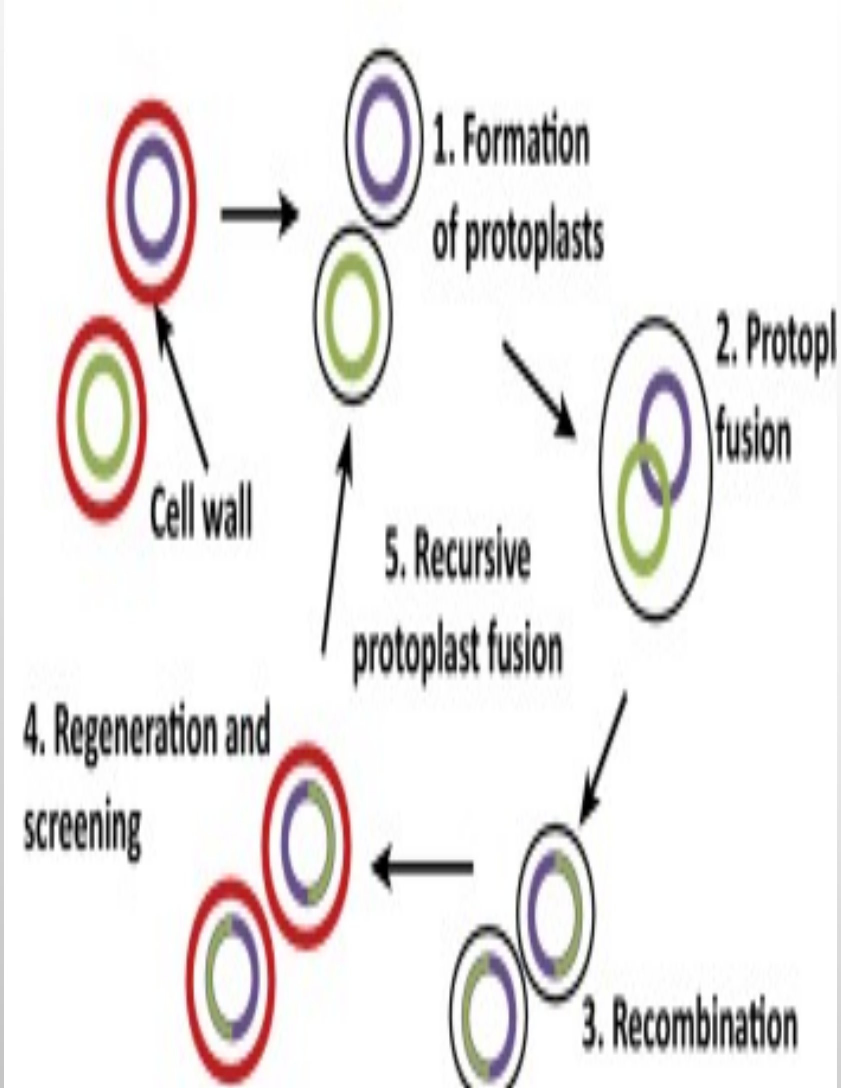
- **Screening for actively fermenting strains**

Actively fermenting isolates were screened for using glucose and maltose sugars according to the methods of Lazarova *et. al.,* (1987).

- **Screening for ethanol tolerance in yeast**

Isolates were screened for ethanol tolerance at varying concentrations (10%, 12%, 15% and 20% (v/v) of absolute ethanol.

Development of Improved Fermenters by Protoplast Fusion

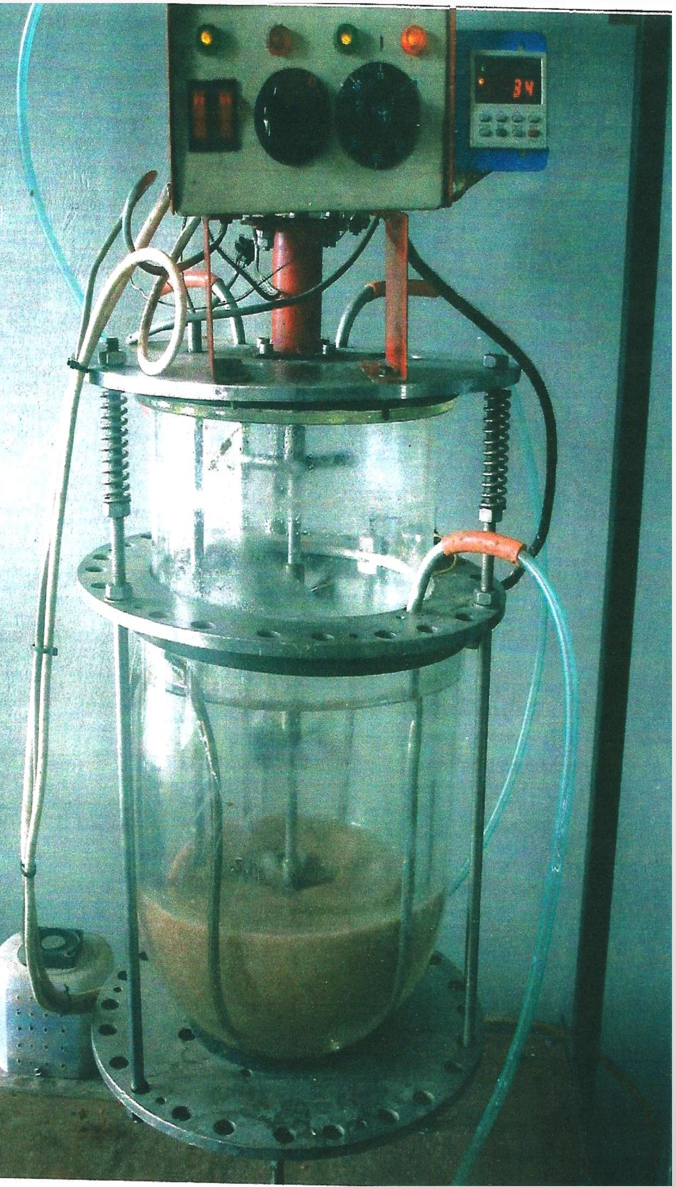


- Improved cultures of *W anomalus* were developed by protoplast fusion technique using the methods of Nwachukwu *et al.* (2008)

- Regenerated colonies were randomly selected and screened for desirable traits such as: (i) **improved fermentation rate, (ii) ethanol tolerance and (iii) volumetric ethanol productivity**

- Stability of selected isolates was determined by cultivating cultures on SDA plates incorporated with **Soy beans (2g/L) as marker** for ethanol tolerance which was assessed for 12 weeks after fusion

SMALL SCALE HYDROLYSIS AND FERMENTATION OF CASSAVA STARCH



- **Extraction of starch from cassava roots**: Starch (30% w/w) was obtained using simple extraction methods of peeling and milling from freshly harvested cassava root (*manihot esculaenta crantz)* weighed 2.5 Kg.

- **Cassava starch hydrolysis:** commercial enzymes, alpha amylase and glucoamylase were used at 4% (v/v) each for the liquefaction and saccharification of the gelatinized starch respectively in a 10L glass double jacketed bioreactor.

- **Fermentation:** subsequent fermentation of the hydrolysed starch was done in an aspirator jar at 30^0c and completed in less than 48h The selected fusant yeast isolates was used at an initial innoculum concentration of 10% (v/v).

ECONOMIC ANALYSIS

- The analysis was done on an industrial capacity of ethanol from a tonne of cassava starch using straight line depreciation for all fixed assets
- Straight line depreciation = (acquisition – salvage price) / useful life
- Salvage value for all fixed assets was assumed as zero
- Production cycle was taken as 2days hence 15 cycles per month and 180 cycles/year

RESULTS AND DISCUSSION

Table 1: Screening for Rate of Fermentation by *W anomalus* from Palm Wine

ISOLATES	Time taken for Glucose Fermentation (h)	Time taken for Maltose Fermentation (h)
W1	6	18
W2	5	10
W3	7	24
W4	8	20
W5	10	26
W6	6	8
W7	6	15
W8	12	24
W9	10	24

* - Selected Parents

Figure 1: Ethanol Tolerance of *W. anomalus* Isolated from Palm Wine

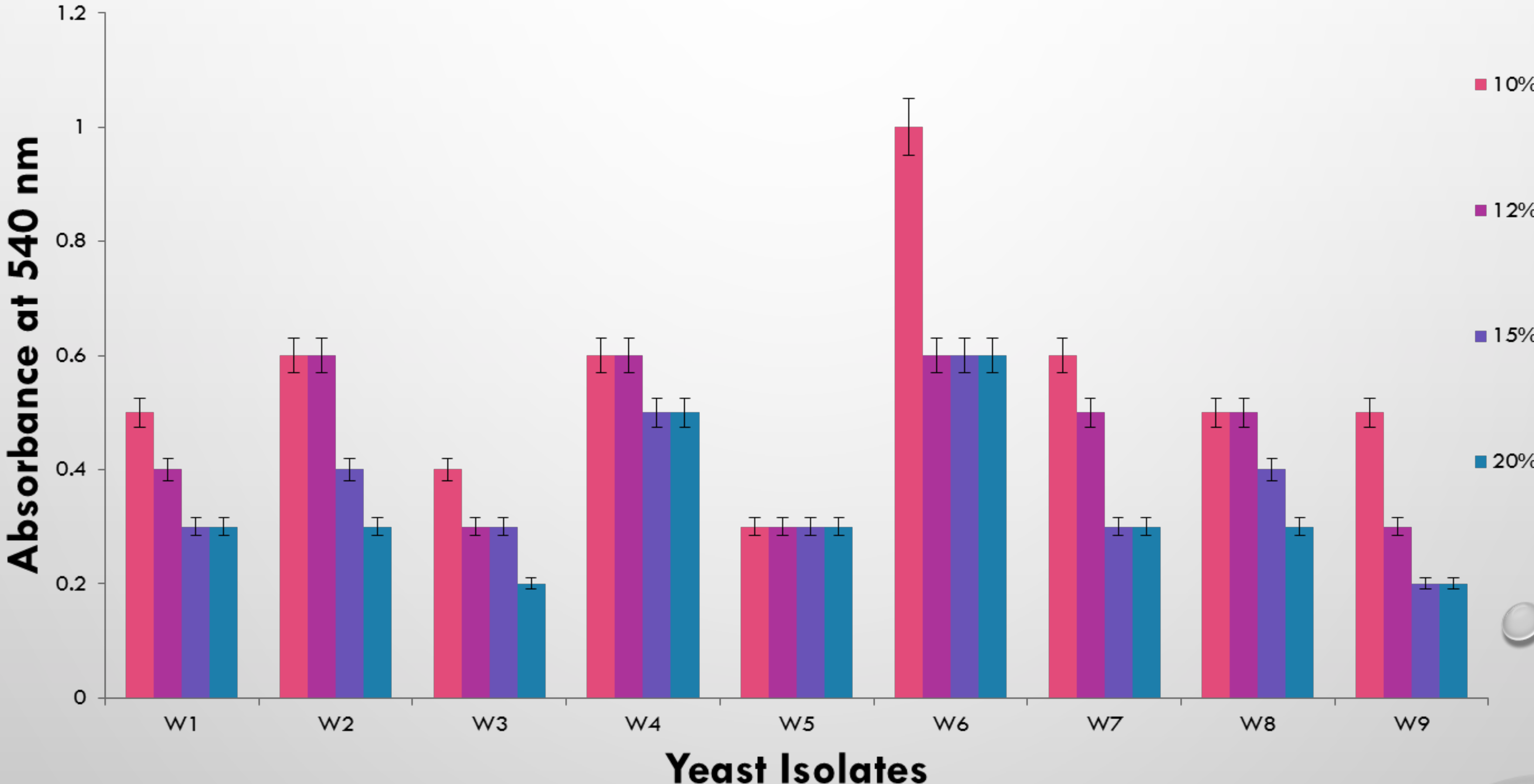


Table 2: Screening for Improved Rate of Sugar Fermentation in Selected Fusants and Parent *W anomalus*

ISOLATES	Time taken for Glucose Fermentation (h)	Time taken for Maltose Fermentation (h)
W2	5	6
W6	10	8
F4	6	8
F5	6	10
F7	5	8
*F10	6	6
F14	5	10
F15	6	8
F18	6	8

*** - Selected Fusants**

Figure 2: Ethanol Tolerance of Regenerated Fusants

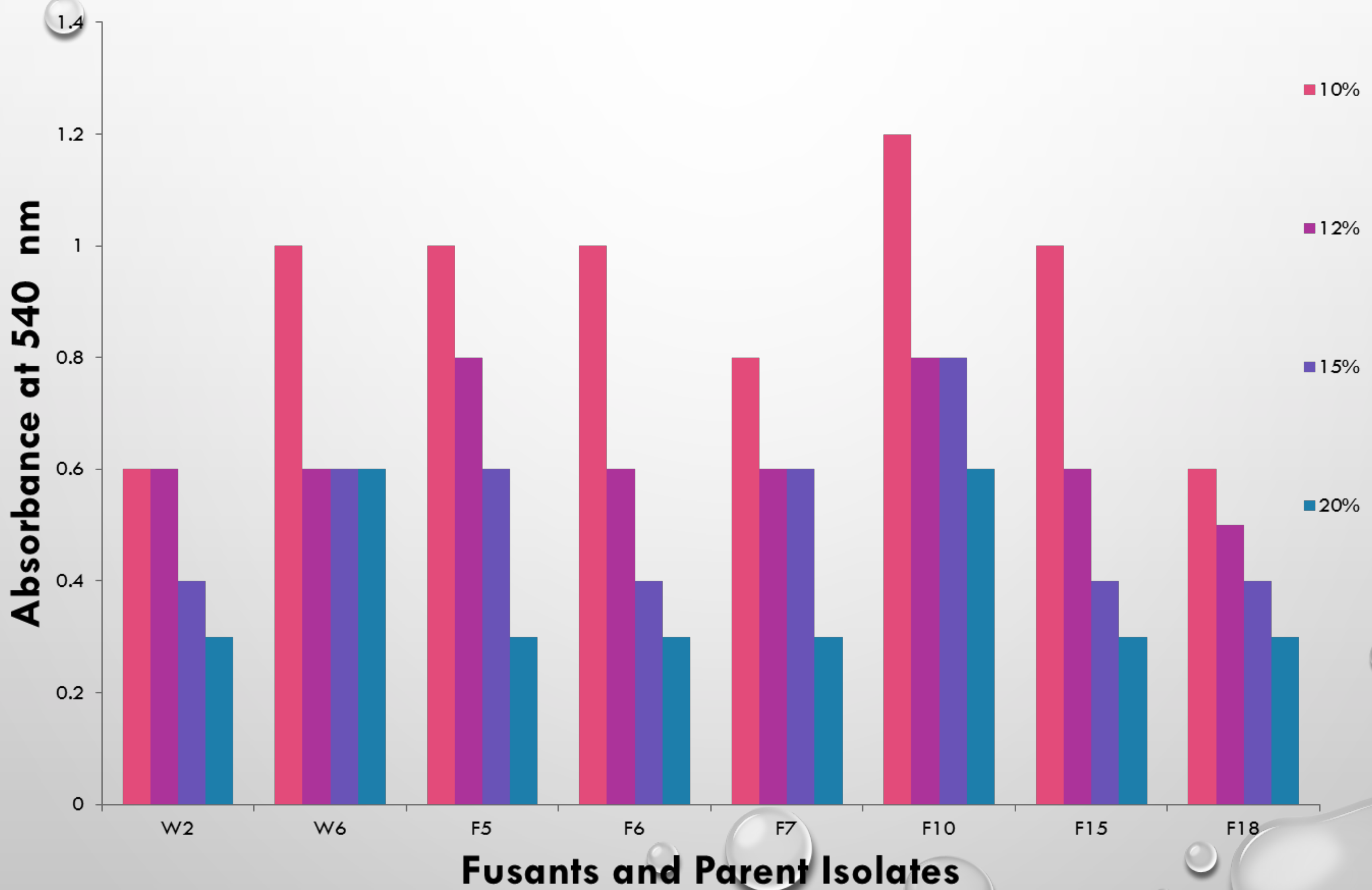


Figure 3: Volumetric Ethanol Prductivities of Parents and Regenerated Fusants

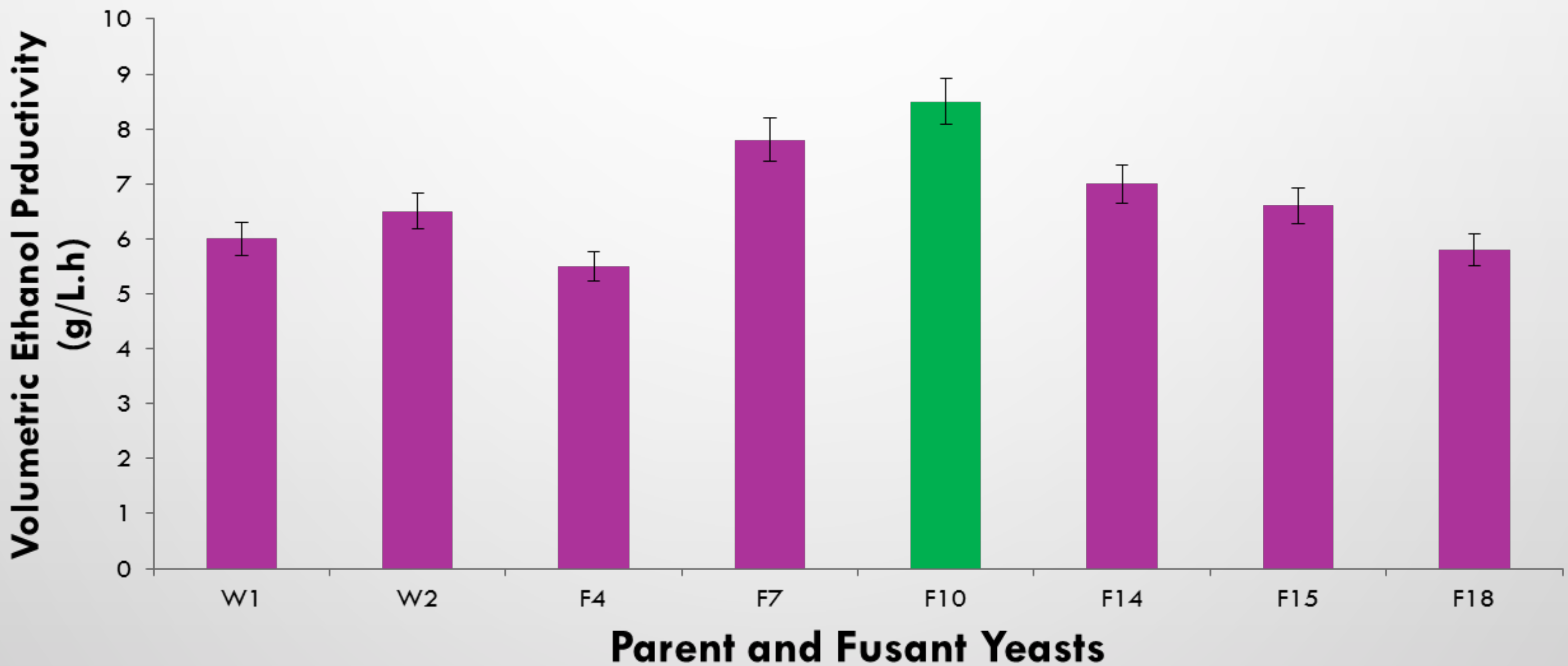
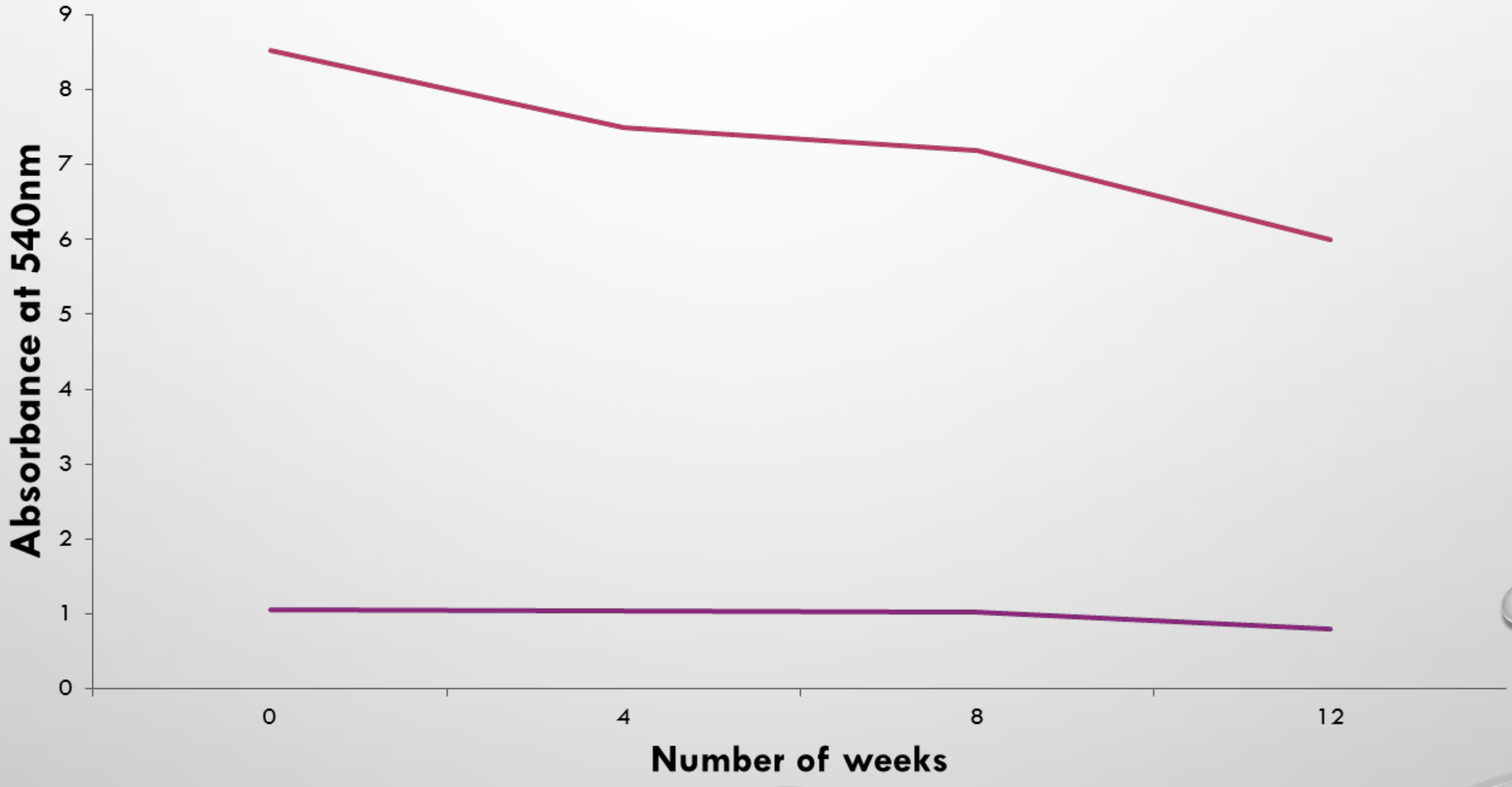


Figure 4: Stability Studies of Selected Fusants



- After a 2-day fermentation of starch extracted from about 2.5kg wet wt. Of cassava, 1.5L of 78% (v/v) ethanol was obtained by distillation.

- Using straight line depreciation, the economic viability and profitability of this project on a commercial scale is illustrated in the tables below and hereby discussed.

ANALYSIS OF ALL FIXED ASSETS

FIXED ASSETS	ACQUISITION PRICE (₦)	LIFE SPAN (YEARS)	COST/TONNE (₦)
BIOREACTOR	1800,000	15	120,000
INCUBATOR	285,000	10	28,500
OVEN	250,000	15	16666.67
AUTOCLAVE	165,000	20	8,250
MILLING MACHINE	200,000	15	13,333
DISTILLATION UNIT	2,000,000	20	100,000
TOTAL			286,749.67

COST OF VARIABLE ASSETS

VARIABLE ASSETS	COST/ CYCLE (₦)
CASSAVA TUBER	13,500
ENZYMES	10,000
MISCELLANEOUS	20,000
ENERGY	10,000
TOTAL	**53,500**

ANALYSIS BASED ON EXPERIMENTAL RESULTS FROM 2.5KG OF CASSAVA TUBER

- TOTAL VARIABLE COST/CYCLE = ₦ 53,500.00
- TOTAL VARIABLE COST/YEAR = ₦ 9,630,000.00
- **TOTAL COST OF ALL ASSETS/YEAR = ₦ 9,916,749.67.00**

- 2.5KG CASSAVA PRODUCED 1.5L OF ETHANOL.
- 1 TONE CASSAVA WILL PRODUCE 600L OF 75% (V) ETHANOL/CYCLE
- THIS TRANSLATES TO 468L OF ABSOLUTE ETHANOL/ CYCLE
- PRODUCTION CYCLE CALCULATED AS 2DAYS= 180 CYCLES/YEAR
- VOLUME OF ETHANOL PRODUCED/YEAR = 824,240L
- CURRENT MARKET VALUE OF ETHANOL =#138.86/L
- **EXPECTED REVENUE = ₦ 11,697,566.40/YEAR**

- PROFIT MARGIN PER ANNUM STANDS AT **#1,780,816.73/TONE OF CASSAVA**

Conclusion

- Improved varieties of fermenters were developed from *W. anomlaus* using a simple less expensive technique of protoplast fusion.

- . The use of cassava starch hydrolysates in production of ethanol, adds value to the relatively low cost crop as ethanol is in high demand for domestic and industrial purposes.

- the bioethanol from cassava starch production process using simple techniques friendly to a growing economy like ours, is not only viable but profitable.

THANKS FOR LISTENIN G