Comparison of ethanol production from cassava chips by fermentation using five yeast strains

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Abstract

Although dried cassava chips are promising for production of fuel ethanol, relatively little has been done to optimize conditions for fermentation of this substrate. Studies were carried out to compare five yeast strains for ethanol production from cassava chips by fermentation. At the initial substrate concentration of 15%, *Candida krusei* strain TISTR 5624 optimally utilized reducing sugars within 24 h, with a theoretical yield of 98.66 %. Similar results were obtained with *Saccharomycese cerevisiae* strains TISTR 5027, TISTR 5596 and *Saccharomyces carlsbergensis* strain TISTR 5018. *Candida tropicalis* strain TISTR 5087 required 48 h to achieve equivalent yields. At 20% substrate, sugars were incompletely utilized by all strains tested, although maximal ethanol exceed 12% . At 25% substrate, both sugar utilization and ethanol production were severely impaired. Results provide useful guidelines for ethanol production from cassava chips by fermentation.

Keywords: *cassava roots, ethanol, yeast strains*

1. Introduction

Cassava (*Manihot esculenta* Crantz) is consumed as a dietary source of energy by some 500 million people (Food and Agricultural Organization of the United States [FAO], 2000). Nevertheless, the low protein content and absence of gluten in cassava are considered disadvantegeous for its use in food products, especially in those where elasticity of the dough is essential for product quality. Root and tuber crops usually contain 70-80% water, 16-24% starch and less than 4% protein and lipids (Hoover, 2001). The technology of ethanol production from starch materials has been well defined but could be improved by introducing systems which require less energy. Ziska, Runion, Tomecek, Prior, Torbet, and Sicher (2009) suggest that root crops have greater potential than corn grains as ethanol sources. An assessment of net energy and supply potentials was also performed to evaluate the potential for using cassava to produce fuel ethanol in Thailand (Nguyen, Gheewala, & Garivait, 2007). The cassava fuel ethanol (CFE) system involves three main segments: cassava cultivation including processing, ethanol conversion and transportation. The CFE system in Thailand was proven to be more energy efficient than CFE in China and corn ethanol in the United States. In Thailand, three sources of carbohydrate feed stocks, namely sugar cane, sugar molasses and cassava root, have been evaluated for their potential for ethanol production by simultaneous saccharification and fermentation (SSF). The results suggest that cassava roots transformed to dried chips are the most suitable raw material for ethanol production, with the production cost and time minimized via simultaneous saccharification and fermentation. The production time was 25% faster than the conventional process, i.e. sequential saccharification and fermentation (Keawsompong, Piyachomkwan, Walapatit, Rodjanaridpiched, & Sriroth, 2009). Another study on ethanol production from cassava mash showed that high viscosity caused resistance to solid-liquid separation and lower fermentation efficiency (Srikanta, Jaleel, Ghildyal, & Lonsane, 1992). High viscosity causes several handling difficulties during processes and leads to incomplete hydrolysis of starch to fermentable sugar (Wang, Bean, McLaren, Seib, Madl, & Tuinstra, 2008; Ingledew, Thomas, Hynes, & McLeod, 1999). The addition of water can reduce mash viscosity, however, the concentration of fermentable sugars is decreased by dilution, and more energy is required for water evaporation (Srichuwong, Fujiwara, Wang, Seyama, Shiroma, & Arakane, 2009). On the other hand, suitable viscosity can be achieved by the enzymatic dissociation of cell-wall.

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In this study, cassava chips of Huay Bong 80 (starch content ≈ 89% dry basis) were investigated. After liquefied starch was converted to maltodextrins by treatment with α-amylase at 90 ˚C, pH 6. Fermentation was performed at 30˚C with the simultaneous addition of glucoamylase and yeast. The aim of this study is to enhance the efficiency of ethanol production by comparing the fermentation performance of five yeast strains over time using different concentrations of cassava substrate. Five yeast strains were selected from wine yeast (*S. cerevisiae* strains TISTR 5596), active dry yeast (*Saccharomyces cerevisiae*) strains TISTR 5027, fermented cassava, alcohol distillery (*Candida krusei* strain TISTR 5624, *Saccharomyces carlsbergensis* strain TISTR 5018) and cassava starch (*Candida tropicalis* strain TISTR 5087). Cassava mashes with suitable concentrations were prepared by pretreatment with α-amylase and glucoamylase. The optimum parameters were regressed by statistical analysis.

2. Material and methods

2.1 Microorganisms

Candida krusei strain TISTR 5624; *Candida tropicalis* strain TISTR 5087; *Saccharomyces cerevisiae* strains TISTR 5027, TISTR 5596 and *Saccharomyces carlsbergensis* strain TISTR 5018 were obtained from Thailand Institution of Scientific and Technological Research (TISTR), Bangkok, Thailand. The strains were maintained on YM agar (5 g peptone/l, 3 g yeast extract/l, 3 g malt extract/l, 10 g glucose/l and 15 g agar/l). Active cultures for inoculation were prepared by growing yeasts on a rotary shaker at 150 rpm, 30˚ C for 24 hours.

2.2 Materials

Cassava root of the cultivar Huay Bong 80 were obtained from the Thai Tapioca Development Institute. Fresh tubers (unpeeled) were washed, cut into small pieces, dried for 2-3 days by sunlight until the moisture content was 6-7 % on a dry weight basis. Dried cassava chips were hammer milled to fine particles before mashing. The chemical composition of cassava mash was determined as followed (AOAC, 1995). Starch content was determined using the Titrimetric Method. Protein, fat, fiber, moisture, and ash content were determined according to a method of AOAC (*ibid.)*. Ash content was determined by combustion at 550˚C. Termanyl (thermo-stable α-amylase) was procured from Novo Industries, Denmark. The α-amylase activity was quantified following the method of Bernfeld (Bernfeld, 2008). Maximum α-amylase activity of 2,243 units per ml of the extract (1 unit = 1 mg reducing sugars liberated during 15 min incubation at 30˚C) and glucoamylase activity of 1,137 units per ml were used for the study. These enzyme preparations were used without further purification.

2.3 Liquefaction

The different cassava mash concentrations were macerated with 0.5µl/g α-amylase pH 6 and incubated at 90˚C for 1 h in a water bath incubator. The pretreated mash was cooled to 60˚C and macerated with 1 µl/g glucoamylase pH 4.5 for 2 h. After liquefaction**,** the mash was cooled at room temperature before subsequent fermentation. Reducing sugar content was quantified according to the dinitrosalicylic acid (DNS) Method (Miller, 1959), relative to a glucose reference curve. All results are reported as the mean of three replications. Dextrose Equivalent (DE) value was estimated as DE = (reducing sugars) x 100/(total dry matter)

2.4 Fermentation

Fermentation experiments were carried out in sterilized 250 ml Erlenmeyer flasks containing different cassava mash concentrations, with 0.2% peptone, 0.2% yeast extract, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.2% $(NH_4)_2HPO_4$. Cell densities were measured on a spectrophotometer at 520 nm. The optical density is also a function of cell morphology such as size and shape. Consequently, an independent calibration curve was required for each condition. The number of viable yeast cells was estimated using a haemacytometer. The logarithm of viable cells was correlated to fermenter optical density measurements. For all experiments, 10% yeast culture was inoculated to yield an optical density of approximately 1.0 at 520 nm. Dissolved solids content in fermentations was estimated as the percentage of sugar by weight in solution (%, Brix). Each experiment was carried out in triplicate. The fermentation efficiency was calculated based on the total available glucose. The theoretical yield of 1 g glucose is 0.511 g ethanol. Ethanol concentration was measured by an Ebulliometer.

3. Results and discussion

3.1 Chemical composition of cassava flour

The chemical composition of cassava flour is shown in Table 1. The most abundant component was starch at 89%. Moisture content, protein, fat and fiber were 6.12%, 0.77%, 0.14% and 1.85%, respectively. The analysis verifies that this cultivar of cassava root (Huay Bong 80) contains a high level of starch but low levels of protein and moisture.

Table 1 Chemical compositions of cassava (Huay Bong 80) flour.

	Chemicals % (dry matter)
Moisture	6.12 ± 0.07
Protein	0.77 ± 0.01
Starch	89 ± 0.20
Fat	0.14 ± 0.01
Fiber	1.85 ± 0.10
Ash	1.54 ± 0.01

3.2 Growth of yeasts

Cell density at 1 x 10^9 per ml typically yields 1 mg/ml cells or 1 g/l. As a rule of thumb, an optical density of 1 unit corresponds to approximately 1 g/l of dry cells. The optical densities are shown in Figure 1. All five yeast strains attained stationary phase between 9 and 24 h.

3.3 Fermentation

Ethanol fermentations were performed by batch process in which glucoamylase and yeast were added simultaneously.

At an initial cassava mash concentration of 15%, *Candida krusei* strain TISTR 5624 depleted reducing sugars within 24 h, with the production of 8.4 ± 0.1%. *Saccharomyces cerevisiae* strains TISTR 5027 and TISTR 5596 and *S. carlsbergensis* strain TISTR 5018 produced similar results at 24 h. *Candida tropicalis* strain TISTR 5087 utilized sugars more slowly, requiring 48 h to reach 8.6 ± 0.1% ethanol. *Candida krusei* strain TISTR 5624 was best overall, reaching 8.9 ±0.1% ethanol at 24 h) (Figure 2).

At 20% substrate, sugars were incompletely utilized by all strains, although maximal ethanol yields exceeded 12%. Thus, if the final ethanol concentration is more important than the efficiency of sugar utilization, 20% would be the optimal concentration of cassava mash. Again, *Candida tropicalis* strain TISTR 5087 utilized sugars more slowly than other strains tested, reaching a maximum of only 6.4 ± 0.1% ethanol at 48 h (Figure 3).

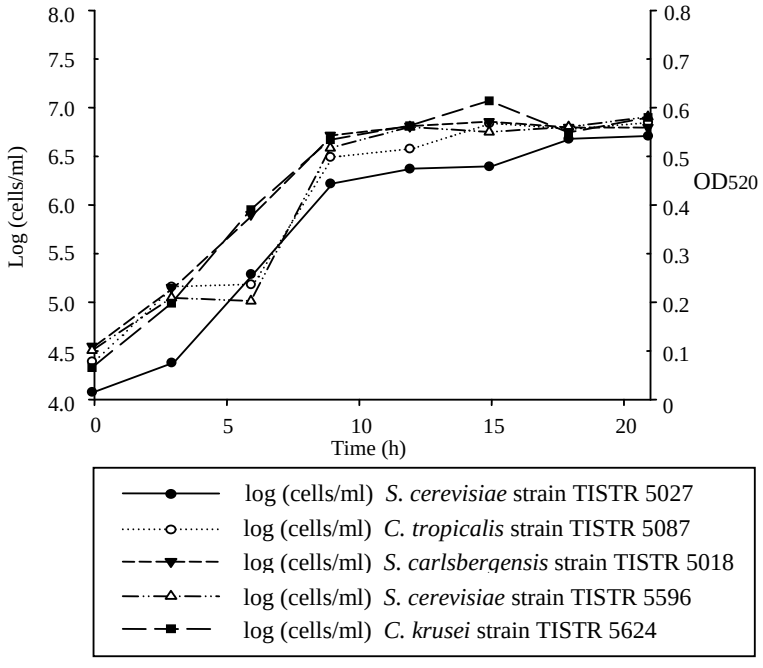

Figure 1 The optical density of five yeast strains (log cells/ml). An optical density of 1 unit (log) corresponds to approximately 1 g/l of dry cell mass.

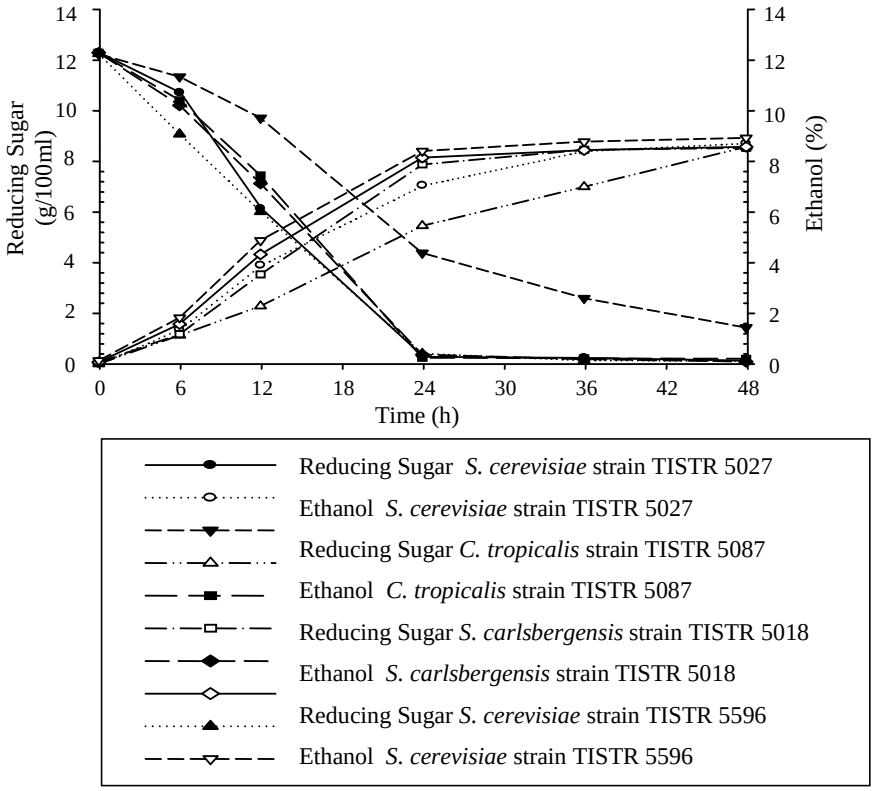

Figure 2 Ethanol production and sugar consumption by five different yeast strains on 15% cassava substrate.

At 25% cassava substrate, sugar utilization was severely impaired for all strains tested (Fig. 4). Ethanol yields were also reduced, with the exception of *S. cerevisiae* strain TISTR 5027, which produced 12.3 ± 0.5% ethanol, equivalent to the amount this strain produced on 20% cassava. Ethanol production efficiencies were lower at 25% substrate concentration due to increased viscousity causing resistance to solid-liquid separation in the fermentor (Wang et al., 2008). Viscosity at high concentrations also caused handling difficulties during processing and may result in incomplete hydrolysis of starch to fermentable sugars (Wang et al., 2008; Ingledew et al., 1999). High cassava concentrations are particularly susceptible to incomplete fermentations because yeast cells are exposed to several stresses, including osmotic pressure. In addition, ethanol concentrations also may be produced in levels toxic to yeasts cells during such fermentations. Osmotic and ethanol stresses result in a loss of cell-viability, growth, and fermentation performance of yeast (Gibson, Lawrence, Leclair, Powell, & Smart, 2007). In addition to osmotic and ethanol stresses, the occurrence of incomplete sacchrification, starch retrogradation, Maillard reactions and other operational losses may contribute to reduced fermentation yields. This often leads to an incomplete fermentation as evidenced by the presence of reducing sugars, a so-called "stuck fermentation". Nutrient limitation of the fermentation medium is also a major factor limiting the rate of fermentation under high gravity (Casey, Magnus, & Ingledew, 1983; Dombek & Ingram, 1986; Jones, & Ingledew, 1994). as yeast cells require sufficient nutrients to survive osmotic stress and maintain metabolic functions.

To calculate the efficiency of the fermentation we calculated the theoretical ethanol yield from starch. Referring to Table 2, in the hydrolysis of starch a water molecule is added across each glycosidic bond, so one gram of completely hydrolyzed starch would give 1.1 g of glucose. From Gay-Lussac's equation the 1.1 g of glucose would theoretically yields 0.567 g of ethanol. This theoretical yield does not take into account ethanol loss due to carbohydrate used for yeast growth and

carbohydrate used in the formation of small amounts of non-ethanol products by the yeast. A simplified biosynthetic Embden-Meyerhof-Parnas pathway from glucose to ethanol is shown in Figure 5. Glycerol and lactic acid are formed in small amounts compared to ethanol synthesis contributing to a yield less than the stoichiometric formation of ethanol from glucose. Allowing for the growth of yeast cells and the formation of fermentation by-products, maximum fermentation efficiency is about 95% of stoichiometric yield (Abouzied & Reddy, 1986) equivalent to 98.66% of the theoretical ethanol yield.

Tables 2 and 3 provide statistical analyses of ethanol production, remaining sugar and theoretical ethanol yield by all five yeast strains on 15% cassava at 24 h. *Candida krusei* strain TISTR 5624 gave the highest ethanol concentration (8.40 ± 0.1%) at 24 h, equivalent to 98.66% of the theoretical ethanol yield. This was not significantly different from results with *S. cerevisiae* strains TISTR 5596. *Candida tropicalis* strain TISTR 5087 was significantly slower than other strains in utilization of sugars for ethanol production. This result corresponds to Rattanachomsri, Tanapongpipat, Eurwilaichitr, and Champreda (2009). *Candida tropicalis* is known to produce ethanol from starch, although at a low rate, due to its production of glucoamylase (Jamai, Ettayebi, Yamani, & Ettayebi, 2007). Jamai *et al*. (2007) reported that starch liquefaction alone was sufficient to drive the fermentation of starch to ethanol by *C. tropicalis* strain YMEC 14, reaching ethanol yields comparable to those obtained by other yeasts using cell surface-engineered *S. cerevisiae* strains that produces both α-amylase and glucoamylase.

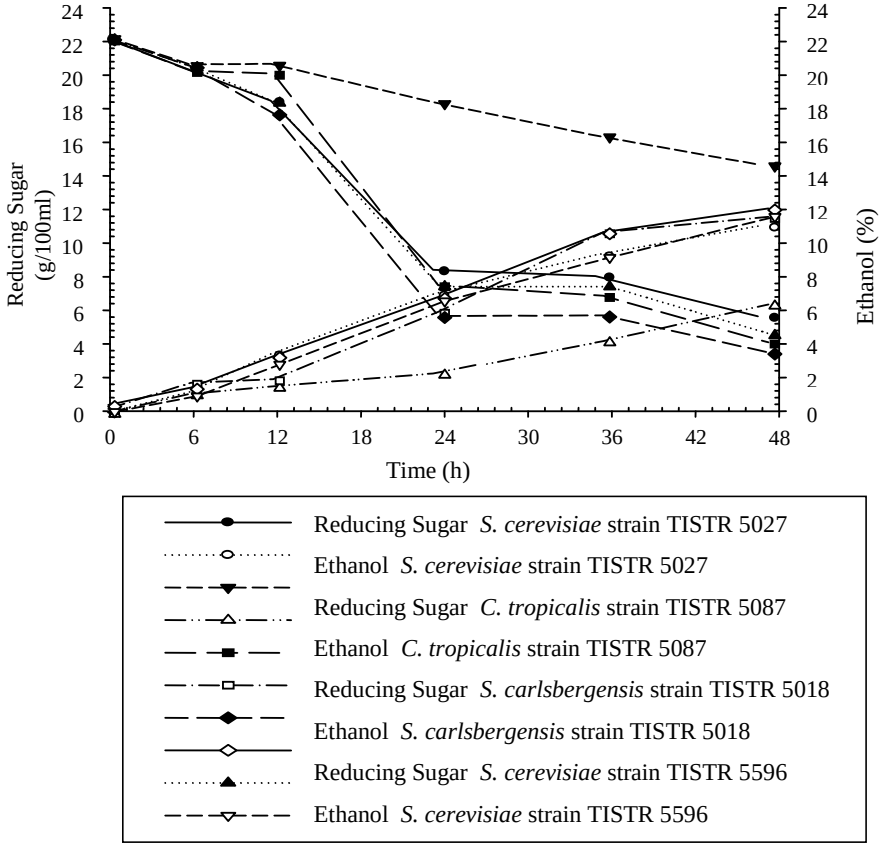

Figure 3 Ethanol production and sugar consumption by five different yeast strains on 20% cassava substrate.

Table 2 Analysis of ethanol production and sugar consumption by five yeast strains on 15% cassava in 24 h

Yeast strains	% Ethanol	Reducing sugar (g/L)	Theoretical ethanol Yield* (%)
S. cerevisiae strain 5027	7.05 ± 0.13^{b}	2.85 ± 0.18^{ab}	82.80
C. tropicalis strain 5087	5.45 ± 0.39^{a}	4.37 ± 1.49^{c}	64.01
S. carlsbergensis strain 5018	7.87 ± 0.06^{c}	2.44 ± 0.20^{a}	92.44
S. cerevisiae strain 5596	8.14 ± 0.07^{cd}	3.62 ± 0.20^{ab}	95.61
C. krusei strain 5624	8.40 ± 0.13^{d}	4.04 ± 0.37^{b}	98.66

$$Theoretical\ ethanol\ yield * (\%) = \left(\frac{mass\ ETOH\ produced}{mass\ carbohydrate\ consumed} \right) x\ 100$$

Table 3 See detailed criteria

Time (h)	% ethanol (v/v)				
	S. cerevisiae strain 5027	*C. tropicalis* strain 5087	*S. carlsbergensis* strain 5018	*S. cerevisiae* strain 5596	*C. krusei* strain 5624
0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.05 ± 0.09^{a}	0.07 ± 0.12^{a}	0.13 ± 0.12^{a}
6	1.37 ± 0.13^{b}	1.13 ± 0.06^{b}	1.17 ± 0.06^{b}	1.57 ± 0.15^{b}	1.82 ± 0.26^{b}
12	3.90 ± 0.10^{c}	2.28 ± 0.08^{c}	3.53 ± 0.08^{c}	4.32 ± 0.13^{c}	4.87 ± 0.15^{c}
24	7.05 ± 0.13^{d}	5.45 ± 0.39^{d}	7.87 ± 0.06^{d}	8.14 ± 0.07^{d}	8.40 ± 0.13^{d}

[a]*Each measurement is the mean of three replications ± one standard deviation. Means within a column with different letters (a,b,c) are significantly different at P < 0.05*

[a,b,c] *Dependent variables:ethanol;different letters refer to significantly different ethanol concentrations at 95% confidence intervals; identical letters refer to insignificantly different ethanol concentrations at 95% confidence intervals*

4. Conclusions

The results demonstrate that yeasts such as *C. krusei* strain TISTR 5624 may be useful for fuel ethanol production from cassava chips by fermentation. Maximal ethanol production (> 12%) was observed at substrate concentration of 20%. Optimal sugar utilization was obtained at an initial substrate concentration of 15%, yielding more than 8.0% ethanol within 24 h. The simple and efficient process described in this study could benefit the cassava root-based ethanol industries without requiring alteration of existing plant equipment.

5. Acknowledgments

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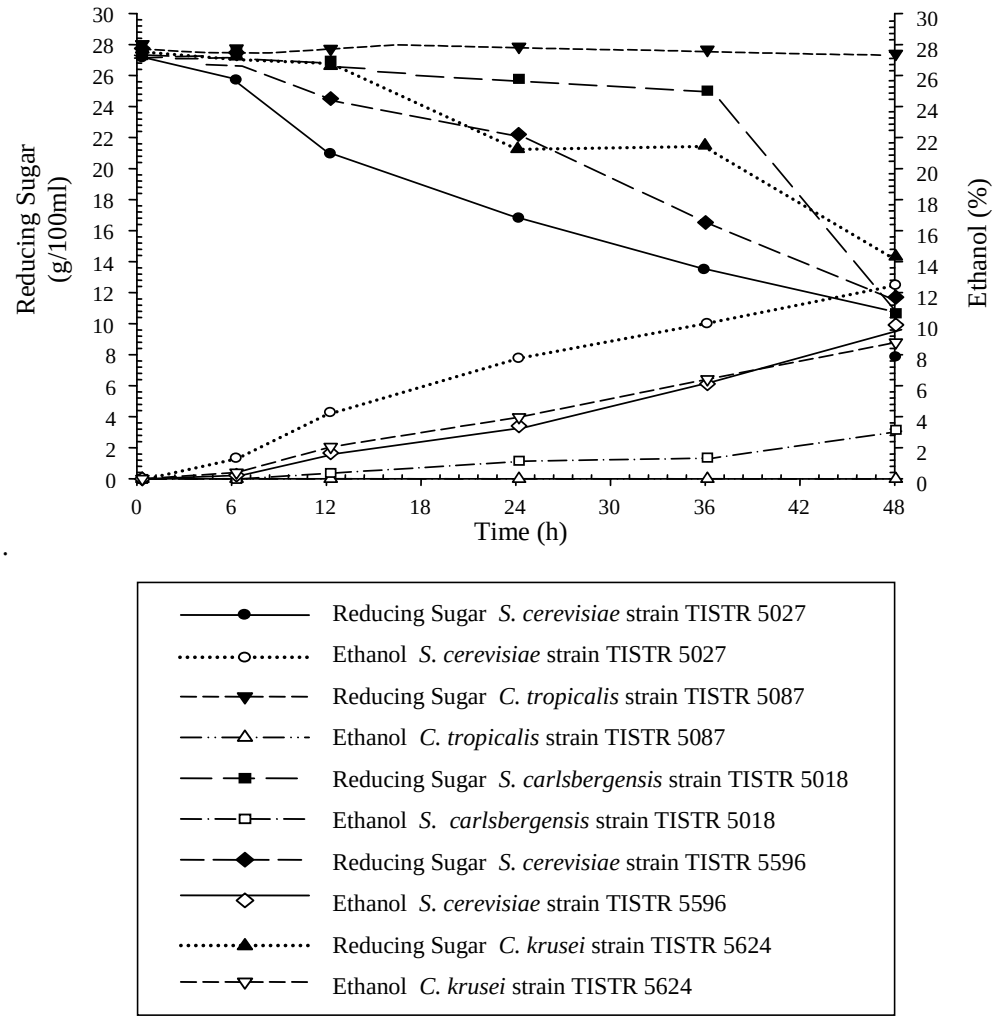

Figure 4 Ethanol production and sugar consumption by five different yeast strains on 25% cassava substrate

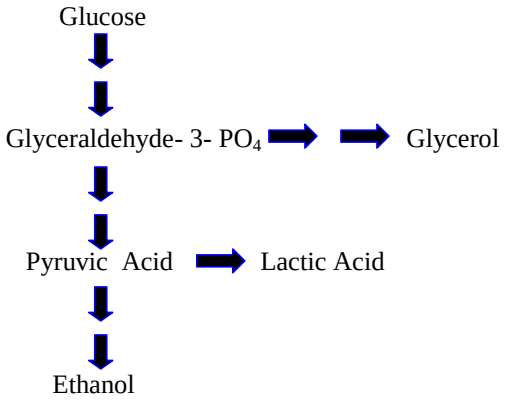

Figure 5 Embden Meyerhof Parnas pathway for ethanol production

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