BIOCONVERSION OF CASSAVA ROOT TO ETHANOL BY CO-CULTIVATION OF AMYLOLYTIC ENZYME PRODUCTION MOULD AND *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

This work was to study the bioethanol production from cassava root using co-fermentation with fungi (*Aspergillus niger*, *Rhizopus oligosporus* and *Trichoderma reesei*) and *Saccharomyces cerevisiae*. The ethanol fermentation from cassava root, one important step in pretreatment is Liquefaction. In this step, the α-amylase enzyme is used to hydrolyze starch in cassava root to get smaller molecules and lower viscosity. For cassava powder (5% w/v) hydrolysis, co-culture of *A. niger* with *R. oligosporus* gave highest reducing sugar of 25.1 g/l at 5th day of cultivation. The highest ethanol of 12.9 g/l was achieved by using *R.oligosporus*, *A. niger* and *T. reesei* for 5 days-fungi fermentation and subsequent ethanol fermentation by co-cultivation with *S. cerevisiae* for 18 hours. Using alpha-amylase liquefaction, *R. oligosporus* fermentation, centrifugation and then *S. cerevisiae* fermentation, ethanol concentration of 45 g/l was found in 24 hours.

KEYWORDS: Bioethanol production, Fermentation, Cassava root, Fungal amylase, *Saccharomyces cerevisiae*

1. Introduction

Several years ago, global oil prices rose steadily since the discovery of oil, there is less and the demand for oil is more current. Oil prices soared a dramatic impact on the world economy in many ways, because oil is a major cost of production. Therefore, finding alternative energy to replace the use of oil, Ethanol is an alternative fuel that is attractive due to the use of ethanol-blended fuel called gasohol still swings the efficiency of the fuel. Most of the ethanol is produced from various raw materials, such as starch crops (wheat, barley, corn and cassava), sugar (molasses or syrup derived from beetroot or sugarcane) and fiber which are by-products of agricultural production (bagasse, corn stalks) [1]. By using different raw materials, different ethanol volumes can be achieved. The ethanol process can be produced from two processes, Chemical Synthesis process which use ethylene to synthesize ethanol and fermentation process by microorganism.

Since cassava is popular to plant in Thailand, the large amount of ethanol is produced from cassava. However, there are two types of cassava which are bitter cassava and sweet cassava. The sweet cassava, which is the type of cassava that people can eat, have low quantity of hydrocyanic acid. On the other hand, the bitter cassava have a lot of hydrocyanic acid which is highly toxic and unfit for human consumption. This type of cassava is used for the processing industry such as starch pellets and alcohol, since it has a high starch content. Moreover, the amount of cassava in Thailand is higher than the demand of people in the country, so cassava can be processed and converted into value-added component such as ethanol. Co-fermentation can simplify the process of saccharification and ethanol fermentation by using co-culture of microorganism.

Saccharomyces cerevisiae is the most common yeasts in fermented ethanol. In alcoholic fermentation uses *S. cerevisiae* strains which will grow fast and resist to high alcohol concentration to ferment [2]. To highlight an advantage of using *S. cerevisiae* is able to grow in a wide temperature range (3-40^{o}C) to withstand high temperatures. Growth of *S. cerevisiae* is at a temperature of 30 °C and pH 3.0-6.0 [3].

The objectives of this research is to determine the production of ethanol from cassava roots by using co-culture of fungi and yeast. The microorganism used in this work were *Aspergillus niger*, *Rhizopus oligosporus* and *Trichoderma reesei* and *Saccharomyces cerevisiae*.

2. Material and Methods

2.1 Materials

Cassava roots "Huay Bong 60" were collected from Nakhon ratchasima, Thailand. The roots were harvested after cultivation for twelve months. Cassava roots were prepared as follow: washing, peeling, chopping, grinding, drying and sieving. The step of grinding was carried out by using grinder screw and drying can be done by sunlight or oven. The size of cassava used in this work was smaller than 100 mesh.

2.2 Microorganism and Inoculum

Aspergillus niger, *Rhizopus oligosporus* and *Trichoderma reesei* cultured in the Sabouraud agar (40 g/l glucose, 10 g/l peptone and 22 g/l agar) and incubated in an incubator at 30^{o}C for 7 days. *Saccharomyces cerevisiae* cultured in YM medium (5 g/l peptone, 3 g/l yeast extract, 3 g/l malt extract and 10 g/l glucose) and incubated at 30^{o}C for 2 days.

2.3 Fermentation

Fermentation in aerobic conditions. Solid-state fermentation were conducted in 250 ml Erlenmeyer flask, with 100ml of cassava hydrolysate (5% w/v of cassava root) that was liquefied by α-amylase 0.3 mg/$g_{cassava}$ at 90^{o}C for 2 hour. The cassava root was added into the solution of 0.9 g/l DAP, 0.1 g/l urea and 0.5 g/l $MgSO_4.7H_2O$ at pH 5.0 acetate buffer. *A. niger*, *R. oligosporus*, *T. reesei* that were carried out separately in tubes with a cotton-wool filter at 30^{o}C in an incubator for 5, 7 and 9 days. A starter culture of *S. cerevisiae* (10% (v/v)) was prepared by growing in yeast mold (YM) under stationary as well as shaking conditions of growth. Experimental conditions for the co-culture of fungi and *S. cerevisiae* were incubated in an incubator shaker at 100 rpm or stationary and 30^{o}C for 48 hours.

2.4 Analysis

Composition of carbohydrate in cassava root was analyzed by method of AOAC 1990. Reducing sugar content was analyzed by DNS method and the ethanol content measured using a high performance liquid chromatography system (HPLC) (Perkin Elmer series 200), equipped with a Reflective Index Detector. An HPLC column (AMINEX HPX-87H, BioRad) was used with 0.005 M sulfuric acid at a flow rate of 0.6 mL/min. The temperature of the column oven and the refractive index detector were 60 and 40^{o}C, respectively.

3. Results and Discussion

3.1 Composition of cassava root

Cassava root is divided into two equal parts for the preparation of peeled and unpeeled, determine the amount of components, including starch, fat and fiber. As shown in Table 1, it was found that cassava chips had higher starch content. It is used in experiments, While the research by Balagopalan (2002) [4] that the cassava chips are a maximum of 5% fiber and a minimum of 65% starch.

Table 1 Composition of Cassava root

Cassava root	Composition		
	Starch (%)	Fat (%)	Fiber (%)
Peeled	80.67	1.21	0.07
Unpeeled	75.44	1.21	0.54

3.2 The solid-state fermentation

Solid-state fermentation of cassava root by fungi was firstly investigated to use amount of total reducing sugar concentration. Overall, the fungal fermentation with shaking for 5 days in higher than other experiments (Table 2) because of the shaking, the fungus can grow in the solution but the stationary, the fungus can only grow on the surface of the solution. The fungus is also use sugar in growth. Total reducing sugar was gradually decreased while the fungi cell density was increased over the fermentation time. Using *A.niger*, *R. oligosporus* and *T. reesei* in the solid-state fermentation, indicated the addition of co-culture *A. niger* and *R. oligosporus* with shaking for 5 days produced reducing sugar of 25.06 g/l which is higher than the reducing sugar of other experiments (Table 2). This is due to the enzymatic production by *A. niger* and *R. oligosporus* that could produce α-amylase and glucoamylase, respectively [5, 6]. That starch hydrolysis to glucose, liquefaction and saccharification requires α-amylase and glucoamylase, respectively. That consistent with the research by Vaidya et al. (2015) [5] that genus of *Aspergillus* can produce amylase enzyme, while the result of research by Freitas et al. (2014) [6] that *R. oligosporus* was a glucoamylase producer. Cassava root was liquefied with alpha-amylase, inoculated fungi for 5 days and then inoculated S. cerevisiae for 48 hours. The result (figure 1) showed that co-culture of *R.oligosporus* and other fungi and yeast (*S. cerevisiae*) can produce ethanol for the reasons mentioned above. The co-culture of *R.oligosporus*, *A.niger* and *T.reesei* and yeast (S. cerevisiae) can produce 12.9 g/l ethanol in 18 hours, is higher than other experiment.

Table 2 Total reducing sugar obtained from solid-state fermentation

Fungus	Condition	Total Reducing sugar (g/l)		
		5 days	7 days	9 days
A. niger	shaking	20.3	20.6	9.7
	stationary	19.8	22.6	19.9
R. oligosporus	shaking	14.7	1.6	1.2
	stationary	13.2	1.4	0.7
T. reesei	shaking	21.9	22.9	22.7
	stationary	21.0	22.1	22.5
Co-culture (*A. niger* + *R. oligosporus*)	shaking	25.1	11.6	13.9
	stationary	22.5	8.9	11.1
Co-culture (*A. niger* + *T. reesei*)	shaking	10.6	22.8	13.8
	stationary	18.9	11.4	12.7
Co-culture (*R. oligosporus* + *T. reesei*)	shaking	13.2	1.2	1.1
	stationary	21.4	11.0	4.5
Co-culture (*A. niger* + *R. oligosporus* + *T. reesei*)	shaking	10.8	3.6	1.3
	stationary	15.2	12.4	6.3

The solid-state fermentation in 250 ml Erlenmeyer flask, with 15% cassava root concentration containing 100 ml of medium, inoculated with fungus culture and incubated at 30°C with shaking at 100 rpm for 5 days. Then, the broth was centrifuged at 910g for 20 minutes to remove the particulate solids. The filtrate was added 10% (v/v) *Saccharomyces cerevisiae* to carry out for ethanol fermentation for 48 hours. The percentages of reducing sugar consumption during ethanol fermentation were shown in Fig 2.

The percentage of sugar consumption of the fermentation *with R. Oligosporus* and *S. cerevisiae* was higher than the sugar consumption of other experiments, as 93.81% for 48 hours. Therefore, this experiment was used to analyze the ethanol concentration by using HPLC. The results were shown in Fig 3. The highest ethanol concentration of the co-culture fermentation with *R. Oligosporus* and *S. cerevisiae* was 45 g/l for 24 hours. The glucose concentration profile had rapidly consumed for 18h and slowly consumed after 24 hours, at this time the ethanol concentration had the highest concentration and decrease after that. The sugar was consumed for yeast growth and ethanol production, the highest concentration was achieved at about 80% of reducing sugar consumption. During the fermentation process, suggesting the sugar was depleted. As a result, the produced ethanol acts as a carbon source in the fermentation, and it is consumed like sugar. This process is used to explain the decrease of ethanol concentration [7].

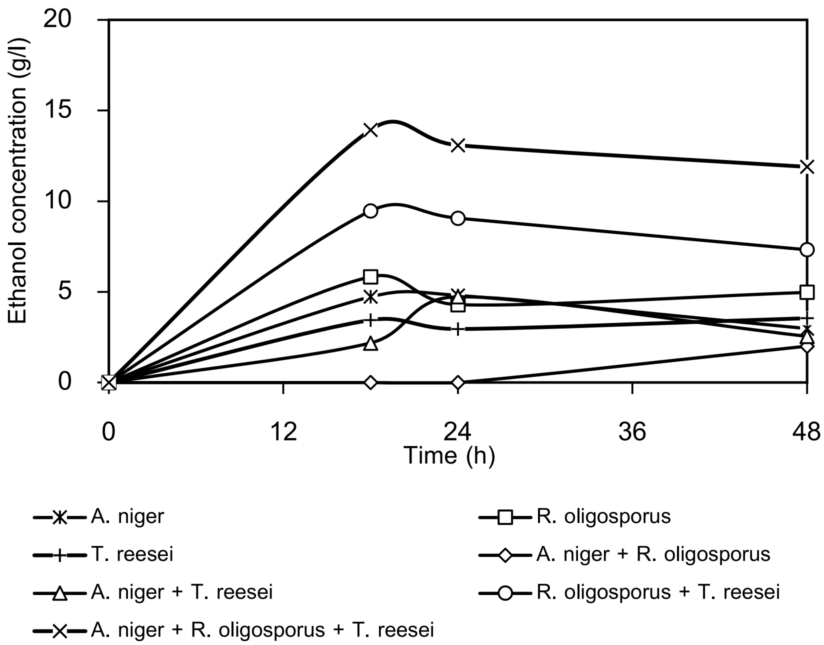

Figure 1 Ethanol concentration obtained from the co-fermentation with fungus and yeast.

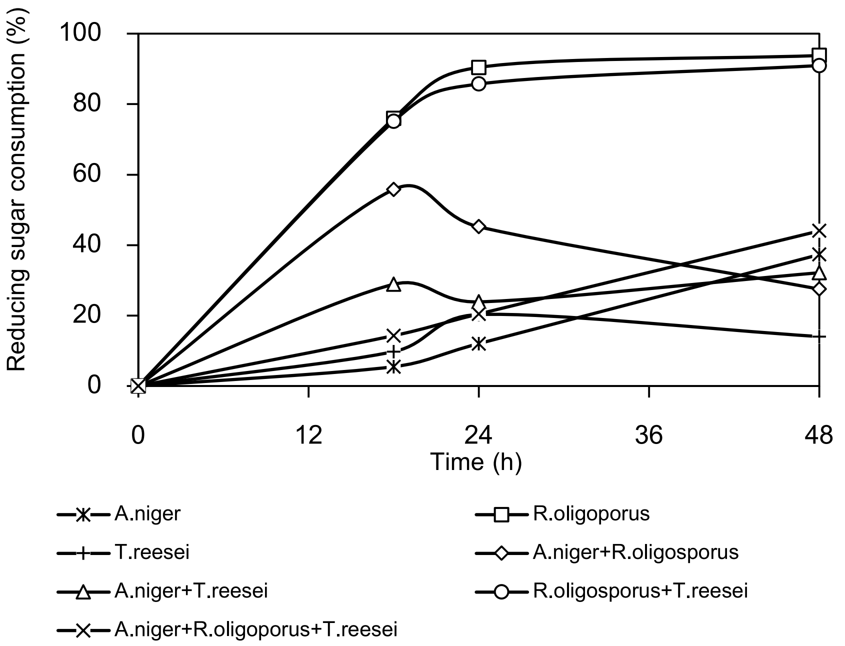

Figure 2 The percentage reducing sugar consumption during ethanol fermentation for 48 hours

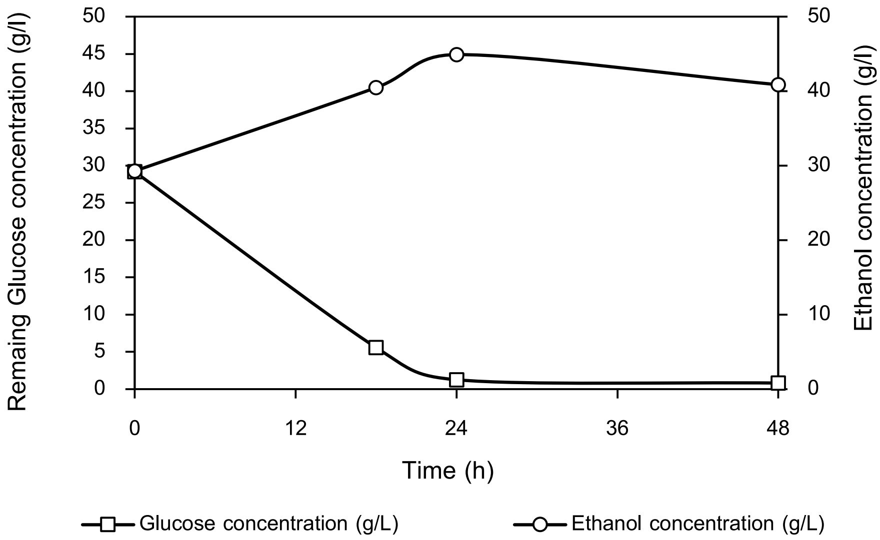

Figure 3 The remaining glucose and ethanol production from fermentation using co-culture *with R. oligosporus* and *S. cerevisiae*.

4. Conclusions

Using alpha-amylase liquefaction, the addition of co-culture *Aspergillus niger* and *Rhizopus oligosporus* on the cassava (5% w/v) hydrolysis gave the highest reducing sugar of 25.1 g/l at 5th day of cultivation with shaking. Using co-culture *Rhizopus oligosporus, Aspergillus niger* and *Trichoderma reesei* for 5 days-fungi fermentation, and added *S. cerevisiae* to co-cultivating fermentation was achieved the highest ethanol concentration as 12.9 g/l for 18 hours. While fermentation of 15%w/v cassava, the addition of *R. oligosporus* was the highest reducing sugar consumption of 93.81% that could produce an ethanol concentration of 45 g/l for 24 hours.

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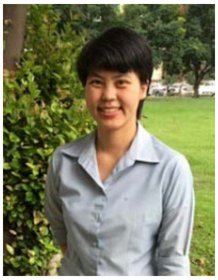

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