Biofuel Production from Cassava Starch by Using Mixed Microorganism Cultures

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บทคัดย่อ

งานวิจัยนี้ได้ศึกษาถึงผลของการผลิตเชื้อเพลิงชีวภาพจากแป้งมันสำปะหลังโดยใช้หัวเชื้อจุลินทรีย์ผสม การทดลองนี้ได้นำลูกแป้งมาจากแหล่งต่าง ๆ เช่น จ.หนองบัวลำภู จ.มหาสารคาม และ จ.ร้อยเอ็ด มาทำการหมักกับแป้งมันสำปะหลัง จากการทดลองพบว่าลูกแป้งที่เก็บจาก จ. หนองบัวลำภู มีปริมาณแอลกอฮอล์สูงที่สุดจึงนำมาใช้เป็นหัวเชื้อในการผลิตหัวเชื้อจุลินทรีย์ผสมจำนวน 4 สูตร จากนั้นนำหัวเชื้อจุลินทรีย์ผสมที่ผลิตขึ้นทั้ง 4 สูตรและนำหัวเชื้อลูกแป้งที่เก็บจาก จ.หนองบัวลำภู มาจำแนกหาปริมาณของเชื้อยีสต์ เชื้อรา แบคทีเรีย และศึกษาคุณสมบัติการผลิตเอทานอลพบว่าหัวเชื้อจุลินทรีย์ผสมสูตรที่ 2 มีปริมาณเชื้อยีสต์สูงที่สุดคือ 6×10^7 โคโลนี/กรัม ของหัวเชื้อจุลินทรีย์ผสม สูตรที่ 3 มีปริมาณเชื้อราสูงที่สุดคือ 3.72×10^7 โคโลนี/กรัม ของหัวเชื้อจุลินทรีย์ผสม และสูตรที่ 1 มีปริมาณเชื้อแบคทีเรียสูงที่สุดคือ 6.1×10^{10} โคโลนี/ กรัม ของหัวเชื้อจุลินทรีย์ผสม ส่วนการผลิตเอทานอลที่สภาวะอุณหภูมิที่ 30°C ค่า pH 7.0, ปริมาณหัวเชื้อจุลินทรีย์ผสมเริ่มต้น 15% และปริมาณแป้งมันสำปะหลังเริ่มต้น 15% และทำการวิเคราะห์หาเปอร์เซ็นต์เอทานอลด้วยเครื่อง GC โดยใช้ Column Porapak Q พบว่า หัวเชื้อจุลินทรีย์ผสมสูตรที่ 4 สามารถผลิตเอทานอลสูงที่สุด คือ 8.67% รองลงมาคือสูตรที่ 2 สูตรที่ 3 และสูตรที่ 1 ซึ่งมีปริมาณเอทานอลเท่ากับ 8.4, 8.3 และ 7.78 % ตามลำดับ เมื่อเปรียบเทียบกับการวิเคราะห์หาเปอร์เซ็นต์เอทานอลโดยใช้ Column PEG พบว่าหัวเชื้อจุลินทรีย์ผสมที่

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ผลิตขึ้นมาใหม่ในสูตรที่ 4 ยังคงให้ปริมาณเปอร์เซ็นต์เอทานอลที่ผลิตได้สูงที่สุด คือ 8.92% รองลงมาคือสูตรที่ 3 สูตรที่ 2 และสูตรที่ 1 ซึ่งมีปริมาณเอทานอลเท่ากับ 8.82, 8.39 และ 8.00 % ตามลำดับ

คำสำคัญ: เอทานอล, แป้งมันสำปะหลัง, หัวเชื้อจุลลินทรีย์ผสม

Abstract

This research was undertaken to determine the effects of ethanol production from cassava starch by using Thai traditional beverage starter or Loog-pang. Loog-pang (dried solid of mixed microorganism culture) was collected from various provinces such as Nong Bua Lam Phu, Maha Sarakham and Roi Et. These cultures were used to ferment cassava starch. In this experiment, the mixed microorganism cultures from Nong Bua Lam Phu province provided the highest percentage of alcohol content. Therefore, it was selected as the original culture for the next following experiments. Four new formulas of mixed microorganism cultures for ethanol production have been developed from Nong Bua Lam Phu province's cultures. The number of fungi, bacteria and yeast populations were counted and the percentage of ethanol produced from cassava starch was also examined in each formula. The result showed that the highest number of yeast (6×10^7 cfu/g of mixed microorganism culture), fungi (3.72×10^7 cfu/g of mixed microorganism culture) and bacteria (6.1×10^{10} cfu/g of mixed microorganism culture) were found in mixed microorganism culture formulas 2, 3 and 1 respectively. In this experiment, the optimal condition for ethanol production was found at 30°C and pH 7.0 when 15% of initial inoculum and 15% cassava starch concentration were added in the fermentation process. Furthermore, the percentage of ethanol was determined by using Porapak Q and PEG Column in GC. The highest percentage of ethanol was detected in the mixed microorganism culture formula 4 in both Porapak Q (8.67%) and PEG (8.92%). In Porapak Q column, 8.4, 8.3 and 7.78 percentage of ethanol were found in the mixed microorganism culture formula 2, 3 and 1 respectively. However, in PEG column 8.82,

8.39 and 8.00 percentage of ethanol were found in the mixed microorganism culture Formula 3, 2 and 1 respectively.

***Keyword**:* Ethanol, Cassava starch, Mixed bacterial cultures

1. Introduction

Bioethanol or ethyl alcohol, a form of renewable energy, has been drawn considerable attention because of the energy and environment security and high energy prices worldwide. Ethanol, an alternative biofuel which is used to blend with gasoline to obtain a product called gasohol, can be produced from various carbohydrate – containing materials by yeast fermentation. Presently, there has been considerable debate about how useful bioethanol will be in replacing gasoline. Ethanol can be produced from many types of feedstocks such as sugar cane, bagasse, sugar beet, sorghum, switch-grass, barley, hemp, kenaf, potatoes, cassava, sunflower, fruit, molasses, corn, stover, grain, wheat, straw, cotton, as well as many types of cellulosic wastes (Martinez, *et. al*. 2018 & Wang, *et. al*. 2012)

In Thailand cassava (*Manihot esculenta* Crantz.) is the third most important economic crops and the average yield is approximately 17 t/ha. Currently, approximately 10 million tons of fresh cassava tubers are consumed annually as a starch staple (Chi, Z. M. *et al*., 2009; Saediman, H. *et al*., 2016) therefore cassava roots are promising to used as the main raw materials for the production of bioethanol. Cassava starch is consisted of unbranched amylose (20 ± 5%) and branched amylopectin (80 ± 5%), both starches can be hydrolyzed enzymatically (in both pure enzymes and amylase-producing microorganisms) and provided glucose and malto-oligosaccharides as the sugar end products (Zuh, 2015). To produce ethanol from cassava, starch is extracted from cassava tubers during manufacturing, grated cassava tubers are separated into starch granules and fibrous residual materials (cassava pulp) by water extraction followed by centrifugation. Initially, starch is

converted to fermentable sugars (mainly glucose) by amylase-producing microorganisms or acid process (Emeka, *et. al*. 2015 & Escaramboni, *et. al*. 2018) The glucose is then fermented to ethanol by yeast. Approximately, 6 kg of fresh roots (25% starch content) or 2.5 kg of dried chips (65% starch content) can produced 1 liter of anhydrous ethanol however the conversion ratio is varied depending on processing efficiency.

Thai traditional beverage starter or Loog-pang (Thai's name) is prepared from alcoholic fermented yeast, starch hydrolysis fungi, herbs, seasoning and glutinous rice. Since Loog-pang contains a combination of alcoholic fermented yeast and starch hydrolysis fungi and it can provide an economically acceptable production for the Thai traditional alcoholic beverage therefore Loog-pang may has shown a great potential for use as a starter in ethanol production processes from cassava starch. Hence, this research was undertaken to examine the effects of ethanol production from cassava starch by using Thai traditional beverage starter. It is expected that the findings of this study will help to develop the appropriate conditions for ethanol production from cassava starch.

2. Objective

To examine the effects of ethanol production from cassava starch by using Thai traditional beverage starter.

3. Research Methodology

3.1 Microorganisms

Loog-pangs (dried solid of mixed microorganism culture) were collected from various provinces in the Northeast of Thailand such as Roi Et, Nong Bua Lam Phu and Maha Sarakham Provinces. These cultures were used to ferment cassava starch. In this experiment, Loog - pang from Nong Bua Lam Phu province gave the highest percentage of alcohol content. Therefore, it has been selected as the initial starter culture for the next following experiments and used as the control.

3.2 Screening medium

YM agar (glucose 10.0 g/l, peptone 5.0 g/l, yeast extract 3.0 g/l, malt extract

3.0 g/l, agar 20.0 g/l and adjusted initial pH to 6.2) was used to screen yeast.

PDA agar (potato 200g/l, dextrose 20 g/l, and agar 15 g/l) was used to screen fungi.

NA agar (beef extract 3 g/l, peptone 5 g/l, agar 15 g/l and adjusted initial pH to 7) was used to screen bacteria.

3.3 Loog-pang (inoculum) preparation for ethanol production from cassava starch

Loog-pang from Nong Bua Lam Phu Province was used as a control and the four new formulation of Loog-pangs were prepared as follows:

Formula 1: glutinous rice flour 85 g, galangal 30 g, spices 30 g and Loog-pang from Nong Bua Lam Phu Province 10g.

Formula 2: glutinous rice flour 100g, dipli 5 g, root of ya -nang 5 g, ehtmolepling red 5 g, odeameroglgm 5 g, lemon grass 5g, cloves 5g, galangal 30g and Loog-pang from Nong Bua Lam Phu Province 10g. Formula 3: glutinousrice flour 400 g, pepper 20g, galangal 20g, dipli 10g, cloves 20g, garlic 20g, licorice 15g and Loog-pang from Nong Bua Lam Phu Province 10g.

Formula 4: glutinous rice flour 100g, galangal 50g, spices 30g, garlic 10g and Loog-pang from Nong Bua Lam Phu Province 10g.

Each formular was mixed thoroughly together then adjusting the moisture content after that made Loog-pang in round shape and left to stand for at least 24 hours in the shade to allow the fugal mycelium grown cover all its surface. After 24 hours, each Loog-pang was dried and kept in the 4°C until used.

3.4 Ethanol production processes

The ethanol production from each Loog-pang formula was studied in relation to temperature, pH, initial cassava starch and Loog-pang concentration. In this experiment the varied conditions are as following: pH 5, 7 and 9, temperature 27°C, 30°C and 37°C, initial cassava starch concentration 5%, 10% and 15% and initial concentration of Loog-pang 5%, 10% and 15%.

3.5 Ethanol production analysis

Ethanol was analyzed by using Porapak Q and PEG columns via gas-chromatograph. The conditions of Porapak

Q column were temperature column 20°C, detector column 220°C, injector column 220°C, stop time = 6, speed time = 3, minimum area = 5000 and attention time = 3. While the conditions of PEG column were column temperature 80°C, detector column 150°C, injector column 120°C, stop time = 3, speed time = 3, minimum area = 1000 and attention time = 3.

3.6 Statistical analysis

The data of percent ethanol from each Loog-pang formula obtained from Porapak Q and PEG columns via gas-chromatograph was analyzed by using SPSS 20 for Windows XP (https://spss-64bits.en.softonic.com).

4. Results and Discussions

4.1 Microbial population

Loog-pangs (dried solid of mixed microorganism culture) were co llected from various provinces in the Northeast of Thailand such as Roi Et, Nong Bua Lam Phu and Maha Sarakham Provinces. These three cultures were used to ferment cassava starch. In this experiment, Loog-pang from Nong Bua Lam Phu province gave the highest percentage of alcohol content. Therefore, it has been selected as the initial starter culture for the next following experiments and used as the control. The four new formulation of Loog-pangs were prepared from Loog-pang from Nong Bua Lam Phu province and number of microbial population in each formula was evaluated and compared. The hightest population number of yeast ($60 \pm 2.28 \times 10^6$ cfu/g), fungi ($37.2 \pm 1.28 \times 10^6$ cfu/g) and bacteria ($6.1 \pm 0.80 \times 10^{10}$ cfu/g) were found in Loog-pang for mula 2, 3 and 1, respectively; Table 1.

Table 1

Number of microbial population in each Loog-pang (colony/g)

Loog-pang sample	Yeast	Mold	Bacteria
Formula 1	$0.97 \pm 1.1 \times 10^6$	$0.71 \pm 1.12 \times 10^6$	$6.1 \pm 0.80 \times 10^{10}$
Formula 2	$60 \pm 2.28 \times 10^6$	$0.09 \pm 1.19 \times 10^6$	$3.78 \pm 1.08 \times 10^{10}$
Formula 3	$40 \pm 1.78 \times 10^6$	$37.2 \pm 1.28 \times 10^6$	$1.48 \pm 1.94 \times 10^{10}$
Formula 4	$2.44 \pm 0.22 \times 10^6$	$1.934 \pm 8.08 \times 10^6$	$4.42 \pm 0.60 \times 10^{10}$
Control (Nong Bua Lamphu)	$0.04 \pm 0.98 \times 10^6$	$0.56 \pm 0.84 \times 10^6$	$1.5 \pm 1.44 \times 10^{10}$

Effect of pH, temperature and inoculum concentration on ethanol yield in 5% cassava starch

The effect of ethanol production processes were carried out in 5% cassava starch concentration at various initial pH (5, 7 and 9), incubation temperature (27°C, 30°C and 37°C) and different initial inoculum concentration (5%, 10% and 15%) of Loog-pang from Nong Bua Lam Phu province (control; Table 2, 3 and 4). By using PEG columns via gas-chromatograph to measure ethanol concentration, the result showed that the highest percent of ethanol (7.60 %; Table 4) was obtained at pH 7 and 30°C when enriched inoculum size into the cassava starch up to 15% while the lowest of percent of ethanol (2.22 %; Table 3) was found in 10% innoculum at pH 9 and 37°C. Under the fermentation conditions tested, optimal conditions to obtain the highest ethanol yield (7.60%) were shown to be at 30°C, pH 7 and 15% of initial inoculum size therefore these conditions were selected to use in the operative fermentation conditions for further studies.

Table 2

Effect of pH, temperature and 5% inoculum concentration on ethanol yield in 5% cassava starch

pH	Temperature (°C)	% Ethanol
5	27	3.68 ± 0.19^{ab}
	30	3.50 ± 0.02^{bc}
	37	3.32 ± 0.02^{cd}
7	27	3.66 ± 0.06^{ab}
	30	3.61 ± 0.11^{ab}
	37	3.02 ± 0.15^{de}
9	27	3.73 ± 0.12^{a}
	30	3.23 ± 0.08^{de}
	37	2.86 ± 0.35^{e}

Means $\pm$ SD in each column with different superscripts indicate statisticant differences ($P<0.05$)

Table 3

Effect of pH, temperature and 10% inoculum concentration on ethanol yield in 5% cassava starch

pH	Temperature (°C)	% Ethanol
5	27	4.64 ± 0.62^{cd}
	30	4.52 ± 0.39^{cd}
	37	4.04 ± 0.02^{d}
7	27	4.86 ± 0.18^{bcd}
	30	4.29 ± 0.13^{cd}
	37	4.93 ± 0.44^{bc}
9	27	5.96 ± 0.42^{a}
	30	5.5 ± 0.11^{ab}
	37	2.22 ± 0.29^{e}

Means $\pm$ SD in each column with different superscripts indicate statisticant differences ($P<0.05$)

Table 4

Effect of pH, temperature and 15% inoculum concentration on ethanol yield in 5% cassava starch

pH	Temperature (°C)	% Ethanol
5	27	7.09 ± 0.18^{a}
	30	7.50 ± 0.48^{a}
	37	4.83 ± 0.01^{c}
7	27	7.17 ± 0.01^{a}
	30	7.60 ± 0.55^{a}
	37	5.36 ± 0.48^{bc}
9	27	5.63 ± 0.04^{b}
	30	4.98 ± 0.03^{bc}
	37	5.41 ± 0.26^{bc}

Means $\pm$ SD in each column with different superscripts indicate statisticant differences ($P<0.05$)

4.2 Effect of Loog-pang (dried solid of mixed microorganism culture) on ethanol yield in different cassava concentration

The four new formulation of Loog-pangs were prepared from Loog-pang from Nong Bua Lam Phu province (control) and these five mixed microorganism cultures were tested for the production of ethanol using cassava starch as the carbon source. Under the fermentation conditions tested above, optimal conditions to obtain the highest ethanol yield (7.60%) were shown to be at 30°C, pH 7 and 15% of initial innoculum size therefore these conditions were selected to use in all the operative ermentation conditions in this studies. To determine the optimum initial cassava starch concentration, fermentation conditions tested were conducted at different initial cassava starch concentration (5, 10 and 15%). It was found that the ethanol production increased when initial cassava starch concentration was arose, the 15% starch concentration was found to be optimum for the highest ethanol production. Comparison of ethanol yields obtained from different formulation of mixed microorganism cultures, the results showed that Loog-pang from Nong Bua Lam Phu Province (control) and mixed microorganism innoculum formula 4 gave insignificant differences in ethanol yields at a confidence level of 95 %. By using

PEG columns via gas-chromatograph to analyze ethanol concentration, ethanol yield from control (Nong Bua Lam Phu Province) and mixed microorganism innoculum formula 4 were 8.82% and 8.92% respectively (Table 5) however ethanol yields were almost similar amount to those found in Porapak Q column (9.03% and 8.67%; Table 6). Among different mixed microorganism innoculum, it was found that ethanol produced from mixed microorganism innoculum formula 1 was slightly lesser yield with is approximately 8% ethanol has been produced from 15% cassava starch concentration however this value was significantly different to the other mixed microorganism cultures.

Table 5

Effect of different mixed microorganism inoculum on ethanol yield in different initial concentration cassava starch (ethanol analysis by HPLC using PEG column)

Cassava starch	Ethanol yield (%)				
	Control	Formula 1	Formula 2	Formula 3	Formula 4
5 %	7.60 ± 0.39^{a}	3.16 ± 0.03^{b}	3.56 ± 0.20^{b}	3.24 ± 0.72^{b}	3.80 ± 0.36^{b}
10 %	8.45 ± 0.27^{a}	6.03 ± 0.25^{b}	6.60 ± 0.20^{b}	6.84 ± 0.75^{b}	6.86 ± 0.41^{b}
15 %	8.82 ± 0.20^{a}	8.00 ± 0.10^{c}	8.39 ± 0.20^{b}	8.82 ± 0.34^{a}	8.92 ± 0.18^{a}

Means $\pm$ SD in each row with different superscripts indicate statisticant differences ($P<0.05$)

Table 6

Effect of different mixed microorganism innoculum on ethanol yield in different initial concentration cassava starch (ethanol analysis by HPLC using Porapak Q column)

Cassava starch	Ethanol yield (%)				
	Control	Formula 1	Formula 2	Formula 3	Formula 4
5 %	7.92 ± 0.40^{a}	3.07 ± 0.25^{b}	3.59 ± 0.16^{b}	3.08 ± 0.73^{b}	3.79 ± 0.22^{b}
10 %	8.52 ± 0.52^{a}	5.74 ± 0.35^{c}	6.25 ± 0.14^{bc}	6.40 ± 0.52^{bc}	6.55 ± 0.14^{b}
15 %	9.03 ± 0.24^{a}	7.78 ± 0.34^{c}	8.48 ± 0.20^{b}	8.38 ± 0.34^{b}	8.67 ± 0.25^{ab}

Means $\pm$ SD in each row with different superscripts indicate statisticant differences ($P<0.05$)

5. Conclusion

Biofuel can be produced from cassava starch by using mixed inoculums. Many factors for biofuel production are necessary in fermentation process such as type of inoculums, inoculums concentration, starch concentration, temperature and pH value. The efficiency of cassava starch conversion to ethanol was achieved by optimization of the culture conditions. In this study, the result found that conversion cassava starch substrate to ethanol can be enhanced by using mixed microorganism innoculum (yeast, mold and bacteria) which consist of starch digesting and sugar-fermenting organisms. In this study the highest ethanol production could be obtained from traditional Loogpang from Nong Bua Lamphu (control) at 9.03% ethanol when measured by HPLC using column Porapak Q. Loogpangs 4, 2, 3 and 1 produced ethanol at 8.67%, 8.48%, 8.34% and 7.78 % respectively in which Loogpangs 4, 2 and 3 were not significant at 95% confidence when comparing ethanol concentration using column PEG. Loogpang 4 could produce highest ethanol at 8.92% and traditional Loogpang (control), Loogpangs 3, 2 and 1 produced ethanol at 8.82%, 8.82%, 8.39% and 8.00 % respectively. Moreover, the optimal conditions for ethanol production were found at 30℃, pH 7.0 when 15% of initial inoculum and 15% cassava starch concentration were added in the fermentation process. Ethanol yield was increased with the increase in cassava starch concentration up to 15%. This result was similar to those reported by Ado, *et. al*., (2009) and who found ethanol yields 3.60 g/100 ml at 8% cassava starch concentration. And also, Ajibola, *et.al*., (2012) found the maximum concentration of ethanol was 5.3% at 10% initial sugar concentration, which gave a sugar conversion efficiency of 37.3%. At pH 7 and temperature 30℃ were favourable for cassava starch conversion and ethanol production these result were concordant which Rhee *et al*., (1984) who found a temperature range between 30°C and 35°C were efficiency of starch conversion to ethanol while at 40℃ the ethanol production by *Z. mobilis* ZM4 was apparently inhibited and the ethanol yields reached a maximum over a wide range of pH 5.0 to 6.5.

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