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Original research article

Optimization of Bioethanol Production from Raw Sugar in Thailand

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ABSTRACT

Since the increasing demand of molasses as raw material for ethanol production in Thailand, the feasibility of applying raw sugar as an alternative raw material for ethanol production is studied in this research. However, although raw sugar has higher sugar content and pure quality than molasses, raw sugar costs are higher than molasses. Thus, it is necessary to evaluate the optimum proportion of raw sugar by considering the value of fermentation efficiency by analyzing the ratio of raw sugar to molasses from 0%:100% to 100%:0%. The results showed that the mixture of raw sugar and molasses in Experiment No.1 at a ratio of 20%:80% gained the highest fermentation efficiency at 82.71%. With the addition of enzyme (Experiment No.2), it would enhance the fermentation efficiency to 84.27% at a ratio of 60%:40%. Moreover, by adding enzyme and ferment nutrients (Experiment No.3) it could enhance the fermentation efficiency to 85.98% at a ratio of 80%:20%. These results indicated that the higher amount of applying raw sugar, the more fermentation efficiency in ethanol production. Furthermore, the economic results shown that even though a ratio of 80%:20% from Experiment No.3 had the highest fermentation efficiency, a ratio of 20%:80% presented the best economic result (profit) with high fermentation efficiency (around 85%). Moreover, when the prices of raw sugar and molasses were changed, the Experiment No.3 had more appropriate operation than Experiment No.1 and No.2, because the Experiment No.3 provieded the best economic results with any conditions.

Keywords: Economic analysis; Ethanol production; Fermentation efficiency; Molasses; Raw sugar

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1. Introduction

As energy demand in transportation sector is now increasing worldwide because continuous depletion of fossil fuels, economic and political crises, and growing concern on environmental safety [1]. Currently, it is found that Thailand needs to rely on importing substantial amount of oil because it cannot increase domestic petroleum production to meet the needs of use and to develop as low carbon society. Thus, the government assigns Ministry of Energy to provide 10-Year Alternative Energy Development Plan (AEDP) to improve the quantity of alternative energy consumption for 25% within 10 years (2012-2021). The report is identified with framework and direction of alternative energy development to stabilize domestic energy sector. One of essential frameworks is to set the target of using ethanol as fuels in transport sector to replace oil-based fuel and it is targeted that ethanol production in 2021 is 9 million liters/day [2]. Under the AEDP, many energy sources such as biomass and transportation fuels like bioethanol from sugarcane molasses are promoted [3].

In present, most domestic ethanol production use cassava and molasses as raw materials and with the target of ethanol production increase, needs on raw materials are increasing as well. However, cassava is agricultural product that can be processed as food, thus, it is not proper to use it as raw material to produce alternative energy. Therefore, ethanol industry development should be mainly focused on ethanol production from molasses. The industry of molasses based ethanol production has production capacity for 87.8% of all ethanol production in Thailand [4,5].

From these situations, it affects needs on increasing quantity of molasses that the price is considerably high. Furthermore, in case of oversupply in sugar market and/or low product quality due to long period storage or defected processes, raw sugar could be a substitute preferable choice for ethanol bioenergy promotion. Especially, the case of factory produces both sugar and ethanol in the area. Accordingly, this research emphasizes on using raw sugar as an alternative to produce ethanol as the price of raw sugar is decreasing [6]. Besides, according to fundamental study, it is found that raw sugar has %brix, purity and fermentable sugar greater than molasses as shown in Table 1 [7]. However, raw sugar prices are higher than molasses. Therefore, it is necessary to study the suitable proportion of raw sugar use by considering from fermentation efficiency and percentage of obtained alcohol. From this point, the suitable proportion of using raw sugar to replace molasses for best fermentation efficiency and highest economic worthiness is evaluated in this research.

Table 1. Properties of molasses and raw sugar [7]

	% Brix	% Purity	% Fermentable Sugar (%FS)
Molasses	80-85	30-35	42-50
Raw sugar	100	98	100

2. Materials and Methods

This work studied alcoholic fermentation by analyzing the proportion of mixture of raw sugar and molasses in 6 conditions including 0%:100%, 20%:80%, 40%:60%, 60%:40%, 80%:20% and 100%:0%. To study possibility of using raw sugar for ethanol production was divided into 3 experiment models as shown in Fig. 1. This research would consider raw sugar with concentration for 60% Brix (60% of dissolved sugar in solution).

Details of each experiment model were as follows

Experiment (Exp.) No.1 : mixture of raw sugar and molasses with specified proportion.

Experiment (Exp.) No.2 : mixture of raw sugar and molasses with specified

proportion added with Invertase (enzyme) to enhance capability of yeasts to consume sugar and convert it as alcohol [8,9].

Experiment (Exp.) No.3 : mixture of raw sugar and molasses with specified proportion added with Invertase and nutrients of Di-ammonium phosphate (DAP) and Urea to increase nutrition of raw sugar [8].

To obtain strong and adequate starter for fermentation, it was necessary to control fermentation conditions in a batch reactor including aeration rate at 0.5-1%, pH at 4.2-4.5 and fermentation temperature at 30-35 ^{o}C [10]. The starter (500 g) was blended continually added with dried yeast or powder yeast (*Saccharomyces cerevisiae*). In the experiment, percentages of fermentable sugar (%FS) of molasses and raw sugar were defined at 48.0%FS and 60%FS, respectively and 11,000 g of total amount of fermentation broth. The quantity of raw materials and chemicals in each experiment was shown in Table 2, 3 and 4. Moreover, the addition of invertase would be considered from %FS which was suitable for converting sucrose into single-molecule sugar. The quantity of DAP and urea would be compared with nutrients in solution in case of fermentation with 0% raw sugar. Nutrients were added so that the solution contains nutrients as same as fermentation with 0% raw sugar.

Fermented sugar solution properties such as pH, %Brix, Specific gravity, %Residual sugar (%RS) and % Alcohol were analyzed in every 3 hours until %RS of the solution was less than 1.50%. The analysis methods were shown in Table 5.

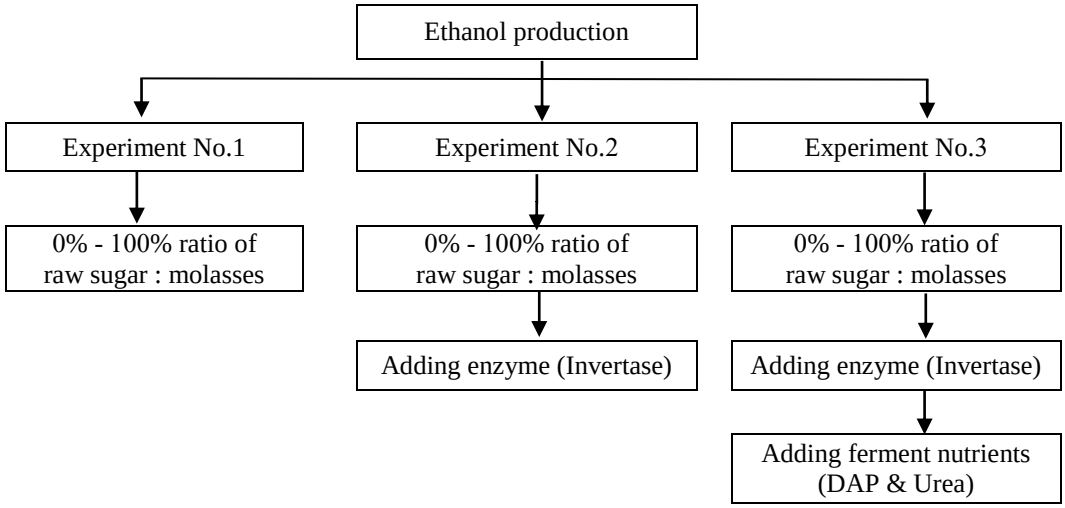

Fig. 1. Experiment models for ethanol production from mixed raw materials of raw sugar and molasses.

Table 2. Quantity of raw sugar and molasses addition in different mixture proportion

% of Raw sugar : Molasses	Raw sugar (g)	Molasses (g)	Water (g)
0 : 100 %	-	3,812	6,688
20 : 80 %	610	3,050	6,840
40 : 60 %	1,220	2,287	6,993
60 : 40 %	1,830	1,525	7,145
80 : 20 %	2,440	762	7,298
100 : 0 %	3,050	-	7,450

Table 3. Quantity of Invertase addition

% of Raw Sugar : Molasses	Invertase (g)
0 : 100 %	-
20 : 80 %	0.067
40 : 60 %	0.134
60 : 40 %	0.201
80 : 20 %	0.268
100 : 0 %	0.335

Table 4. Quantity of DAP and Urea addition

% of Raw sugar : Molasses	Raw sugar (g)	Molasses (g)	Water (g)
0 : 100 %	-	3,812	6,688
20 : 80 %	610	3,050	6,840
40 : 60 %	1,220	2,287	6,993
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80 : 20 %	2,440	762	7,298
100 : 0 %	3,050	-	7,450

Table 5. Fermented solution property measurements

Properties	Analysis methods
pH	pH meter
% Brix	Refractometer
Specific gravity	Hydrometer
% Residual sugar	Tritration with Fehling's Solution using Methylene blue as an indicator
% Alcohol	Ebulliometer

Fermentation efficiency and % alcohol according to theory calculation were

$$Fermentation\ efficiency\ (\%) = \frac{\%Alcohol\ (\ from\ experiment)}{\%Alcohol\ (\ from\ theory)} \times 100\% \quad (1)$$

$$\%Alcohol\ (theory) = \frac{Total\ sugar \times \%FS \times 0.644}{Total\ amount\ of\ broth} \times 100\% \quad (2)$$

where %FS of fermentation broth was defined at 48% and 1 kg of glucose could be converted into ethanol to 0.644 liters.

3. Results and Discussion

This research studied possibility of using raw sugar for ethanol production with 3 experiment models. To measure fermentation efficiency correctly, experimental results would be analyzed for 3 times in each model.

3.1 Fermentation efficiency

The possibility of using raw sugar for ethanol production was evaluated by analyzing % alcohol and fermentation efficiency. The experimental results were shown in Table 6 and found that % alcohol

and ethanol fermentation efficiency prepared from only molasses (Experiment No.1 with 0:100% ratio of raw sugar and molasses) was 8.92% and 83.18%. It was obvious that % alcohol from the experiment was lower than the theory value (10.72%) due to the fact that in fermentation process, some sugar was consumed by yeast to grow and to multiply cells. However, some parts were used to synthesize other products such as carbon dioxide, succinic acid, glycerol and fuel oil etc. Theoretical calculation was not concerned with this part. When 20% raw sugar was mixed with 80% molasses (20:80%) in Experiment No.1 (fermented in as same condition as fermentation with 0% raw sugar), it was found that the decrease of molasses leads to lower fermentation efficiency that % alcohol and fermentation efficiency from raw materials of 20% raw sugar and 80% molasses were 8.87% and 82.71%, respectively. Furthermore, the tendency of % alcohol and fermentation efficiency still continually decreased when adding more raw sugar as yeast could not use raw sugar as food and converted it into alcohol.

Therefore, invertase enzyme was added as in Experiment No.2 to enabled yeast to consumed sugar for growth. The results of Exp.No.2 shown in Table 6 indicated that adding invertase could enhance fermentation efficiency. The experimental results were found that fermentation with 60% raw sugar consisted of higher fermentation efficiency for 84.27% which was slightly higher than fermentation with 0% raw sugar (0:100%) as invertase would change sugar structure that yeast could consume sugar easier. However, as adding invertase would enable yeast to consumed sugar to grow only not to increase nutrients in sugar, when molasses was decreased, the nutrients will also be decreased as raw sugar did not contain nutrients. Thus, the tendency of fermentation efficiency dramatically decreased when proportion of raw sugar was higher than 60%. Therefore, this research studied about the addition of di-ammonium phosphate and urea in fermentation process as in Experiment No.3 to increase nutrients in raw sugar. The experimental results of Exp.No.3 were found that, when nutrients were added, yeasts had sufficient food to grow and that fermentation efficiency was likely to increase when raw sugar proportion was increased.

For the comparison of 3 experiment models, it was found that Experiment No.3 with 80% raw sugar and 20% molasses (80:20%) had highest fermentation efficiency at 85.98% and % alcohol was 9.22% higher than fermentation with 0% raw sugar (0:100%). In addition, when comparing fermentation efficiency from such 3 experiment models with different proportion of raw sugar and molasses as shown in Fig. 2, it found that Experiment No.3, apart from fermentation of raw sugar and molasses in different proportion, was added with enzyme (invertase) and nutrients (di-ammonium phosphate and urea) resulting in the fermentation efficiency higher than fermentation without enzyme and nutrients addition (Experiment No.1) or fermentation added with only enzyme (Experiment No.2).

3.2 Economics analysis

In this research, economic study was evaluated by comparing raw material cost of alcohol production from 3 fermentation process models: Experiment No.1 which was fermentation of raw sugar and molasses in different proportion, Experimental No.2 which was fermentation of raw sugar and molasses added with enzyme (invertase) and Experimental No.3 which was fermentation of raw sugar and molasses added with enzyme and nutrients of di-ammonium phosphate (DAP) and urea.

Table 6. % alcohol and fermentation efficiency from 3 experiment models

% Raw sugar : Molasses	% Alcohol (Theory)	% Alcohol (average)			% Fermentation efficiency (average)		
		Exp.No.1	Exp.No.2	Exp.No.3	Exp.No.1	Exp.No.2	Exp.No.3
0 : 100 %	10.72	8.92	9.02	9.00	83.18	84.11	83.96
20 : 80 %	10.72	8.87	8.82	9.15	82.71	82.25	85.35
40 : 60 %	10.72	8.47	8.88	9.10	78.98	82.87	84.89
60 : 40 %	10.72	8.08	9.03	9.20	75.40	84.27	85.82
80 : 20 %	10.72	8.02	8.60	9.22	74.78	80.22	85.98
100 : 0 %	10.72	7.73	8.50	9.02	72.14	79.29	84.11

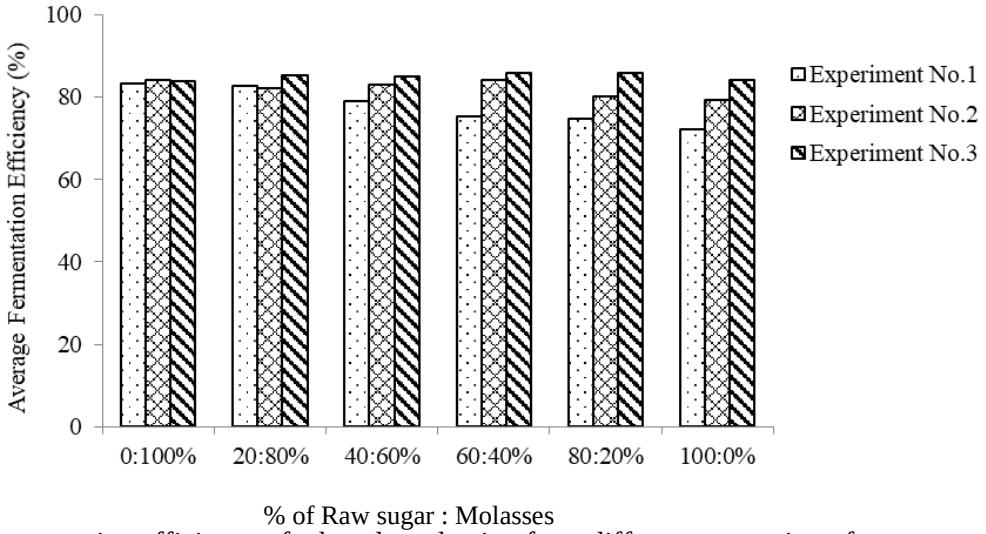

Fig. 2. Fermentation efficiency of ethanol production from different proportion of raw sugar and molasses.

Table 7. Cost of raw materials and chemicals for ethanol production [13]

Raw materials and Chemicals	Cost (THB/kg)
Molasses	5
Raw sugar	14
Invertase enzyme	1,100
DAP	40
Urea	14

The cost relevant to alcohol production comprised two major parts including cost of main raw materials and chemicals as shown in Table 7. The cost of fermentation would be considered from obtained ethanol with alcohol concentration at 8-9% v/v and then concentration would be increased to 99.5% v/v by dehumidification process. Pure ethanol (99.5%) could be blended with gasoline to produce fuel gas known as gasohol [11]. The cost of selling 99.5% alcohol was considered at 23 THB/liter [12].

Furthermore, it is possible that ethanol production cost would change if price of raw sugar and molasses increases or decreases. Therefore, this research was

conducted to study and analyze the return of ethanol production from alternative raw materials (which were raw sugar mixed with molasses in different proportion). In case that the cost of raw sugar and molasses changes, manufacturers can apply this information as guidance to support decision makers on further operations. To analyze suitability of raw material price for ethanol production, it would be considered in terms of main production cost that would possibly change such as raw sugar price changing from 10 to 18 THB/kg and molasses price changing from 2 to 8 THB/kg. Then, returns (profit or loss) would be considered to analyze return trends. It would show the return values in 5 levels with different colors as shown in Table 8 including green showing the profit more than 3 THB/liter, light green representing profit of 1 to 3 THB/liter, yellow indicating breakeven or potential variance of production interval, labor cost and other expenses about 4% per liter of alcohol [14] with profit of (-1) to 1 THB/liter, orange represents acceptable loss if raw sugar cannot be distributed and it is used for production with loss at (-1) to (-1.5) THB/liter, while red shows that when raw sugar and molasses price increases causing significant loss, raw sugar should not be mixed with molasses with loss more than (-1.5) THB/liter.

Details of return result was exampled in Fig.3 which was the study of Experiment No.3 at 20% raw sugar : 80% molasses to analyze returns represented by colors in producing and distributing 99.5%v/v ethanol when prices of raw sugar and molasses were different. From Fig.3 indicating that for Experiment No.3 at 20% raw sugar (20:80%), lower prices of raw sugar and/or molasses produce higher profit (green area). When raw sugar and/or molasses prices increased, the return was likely to decrease (turning from green to light green, yellow and orange) until it made no profit (red).

The comparison of return results of 3 experiment models was shown in Fig.4. It found that when cost of raw sugar and/or molasses increased, ethanol production from mixed raw materials in different proportion would be less profitable (represented by green and light green color). In addition, the results of using raw sugar in different proportion showed the lower profit than fermentation with only molasses (0:100%). However, if it was compared in each experiment model, it was found that Experiment No.3 tended to make more profit as it could notice from wider green and light green area than Experiment No.1 and No.2 at same proportion of raw materials. Furthermore, Experiment No.3 contained yellow area on the right side of the figure indicating that ethanol production from mixed raw materials would not generate loss (but no profit) when cost of molasses was high. The red and orange area (area with loss) of Experiment No.3 was less than the others and it was during the period that cost of molasses was higher than Experiment No.1 and No.2. Conclusively, when price of molasses and raw sugar was increased, Experiment No.3 would make more profit than Experiment No.1 and No.2; in other words, Experiment no.3 was most suitable in economic term to use raw sugar mixed with molasses as alternative raw material of ethanol production. Moreover, it was found that Experiment No.3 at 20% raw sugar (20:80%) was most suitable for real practices because it could make profit even though the cost of raw materials changed. Even though the fermentation efficiency at this experiment (Experiment No.3 with ratio of 20:80% was 85.35%) that was not highest compared to ratio of 80:20% (the efficiency was 85.98%), but this condition (80:20%) may cause great loss. In addition, fermentation efficiency with 85% was appropriate for ethanol production.

Table 8. Guideline to consider return (profit or loss) of ethanol production from raw sugar mixed with molasses in different proportion

Color	Profit (THB/liter)	Definitions
Dark Green (highly satisfactory profit)	3 and above	Highly satisfactory profit
Light Green (acceptable profit)	1 to 3	Acceptable profit
Yellow (breakeven point)	(-1) to 1	Due to production with variance including labor cost and other expenses
Orange (slight loss)	(-1) to (-5)	It is possible to break even (yellow) if production process is improved or the factory has good management or material cost changes
Red (heavy loss)	less than (-5)	Unacceptable loss

20%Raw sugar : 80%Molasses		Price of molasses (THB)												
		2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8
Price of raw sugar (THB)	18	9.14	7.92	6.70	5.48	4.26	3.04	1.81	0.59	-0.63	-1.85	-3.07	-4.30	-5.52
	17.5	9.38	8.16	6.94	5.72	4.50	3.28	2.05	0.83	-0.39	-1.61	-2.83	-4.06	-5.28
	17	9.62	8.40	7.18	5.96	4.74	3.52	2.29	1.07	-0.15	-1.37	-2.59	-3.81	-5.04
	16.5	9.86	8.64	7.42	6.20	4.98	3.76	2.53	1.31	0.09	-1.13	-2.35	-3.57	-4.80
	16	10.10	8.88	7.66	6.44	5.22	4.00	2.77	1.55	0.33	-0.89	-2.11	-3.33	-4.56
	15.5	10.34	9.12	7.90	6.68	5.46	4.24	3.01	1.79	0.57	-0.65	-1.87	-3.09	-4.32
	15	10.58	9.36	8.14	6.92	5.70	4.48	3.25	2.03	0.81	-0.41	-1.63	-2.85	-4.08
	14.5	10.82	9.60	8.38	7.16	5.94	4.72	3.49	2.27	1.05	-0.17	-1.39	-2.61	-3.84
	14	11.06	9.84	8.62	7.40	6.18	4.96	3.73	2.51	1.29	0.07	-1.15	-2.37	-3.60
	13.5	11.30	10.08	8.86	7.64	6.42	5.20	3.97	2.75	1.53	0.31	-0.91	-2.13	-3.36
	13	11.54	10.32	9.10	7.88	6.66	5.44	4.21	2.99	1.77	0.55	-0.67	-1.89	-3.12
	12.5	11.79	10.56	9.34	8.12	6.90	5.68	4.45	3.23	2.01	0.79	-0.43	-1.65	-2.88
	12	12.03	10.80	9.58	8.36	7.14	5.92	4.69	3.47	2.25	1.03	-0.19	-1.41	-2.64
	11.5	12.27	11.04	9.82	8.60	7.38	6.16	4.94	3.71	2.49	1.27	0.05	-1.17	-2.40
	11	12.51	11.28	10.06	8.84	7.62	6.40	5.18	3.95	2.73	1.51	0.29	-0.93	-2.16
	10.5	12.75	11.52	10.30	9.08	7.86	6.64	5.42	4.19	2.97	1.75	0.53	-0.69	-1.91
	10	12.99	11.76	10.54	9.32	8.10	6.88	5.66	4.43	3.21	1.99	0.77	-0.45	-1.67

Fig. 3. The return results of Experiment No.3 at 20% raw sugar and 80% molasses (20:80%).

4. Conclusion

This research studied efficiency of ethanol production with raw sugar as alternative raw material by comparing properties of fermentation efficiency from mixture of raw sugar and molasses in different proportion. The results were found that raw sugar could be used for ethanol production. However, as yeast could not directly consume raw sugar as food, it was necessary to add invertase (enzyme) to enhance it capability to consume raw sugar and convert it into alcohol. Furthermore, nutrients of di-ammonium phosphate and urea were supplemented as raw sugar was non-nutritive. The study results were found that mixture of 80% raw sugar and 20% molasses had highest fermentation efficiency of 85.98% which was higher than fermentation with only molasses whose efficiency was only 83.96%.

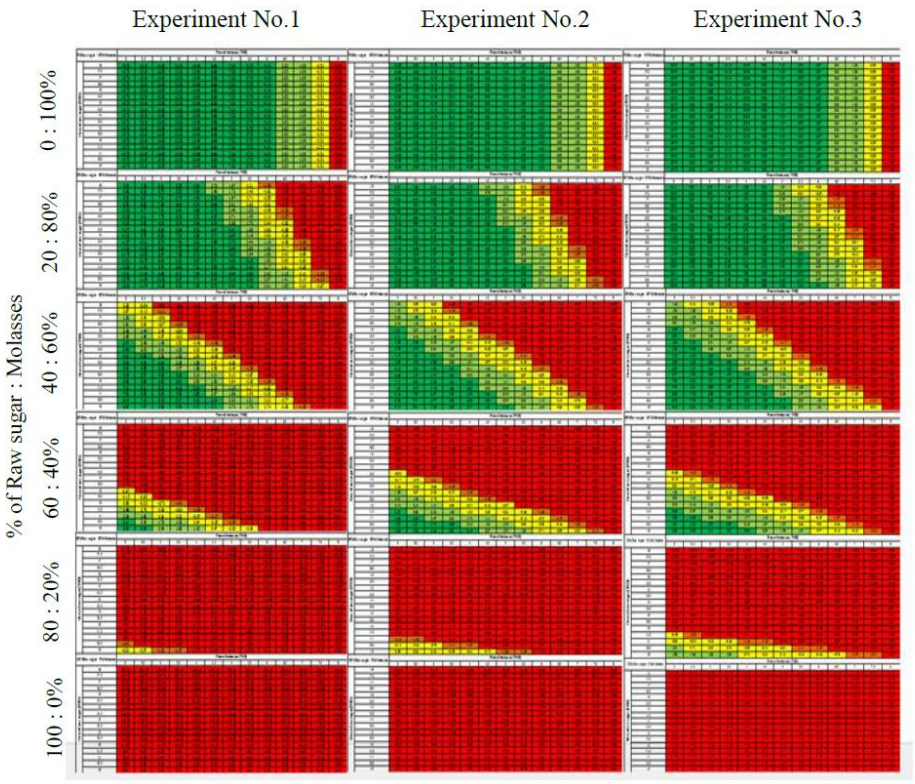

Fig. 4. Comparison of economic analysis in 3 experiment models with different proportion of raw sugar and molasses.

As raw sugar has higher purity than molasses, it can be used to produce ethanol with high fermentation efficiency. However, the price of raw sugar is higher than molasses and additional expense from supplementing invertase and nutrients. Therefore, profit evaluation should be studied to ensure that other than practical possibility in this research using raw sugar is economically possible as one of guidelines to enhance ethanol production capacity according to AEDP. As the price of raw sugar and molasses is likely to increase or decrease, this research was conducted to analyze the return (profit or loss) for ethanol production from three different experiment models when the price of raw sugar and molasses changed from current price. The study results could be concluded that when price of raw sugar and molasses increases, ethanol product would generate lower profit or cause loss. However, when the price of raw sugar and molasses became high, the Experiment No.3 was most economically suitable because it possibly brought higher profit than Experiment No.1 and No.2. Therefore, if necessary to used mixed raw materials of raw sugar and molasses as alternative material for ethanol production such as sugar refinery plant (especially the case of sugar plant incorporated with ethanol plant) that had problems about product quality or lower price of raw sugar, the Experiment No.3 would be most applicable to use raw sugar as alternative of ethanol production to solve the problems of profit loss from defective raw sugar.

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References

[1] Zabed H, Faruq G, Sahu JN, Azirun MS, Hashom R, Boyce AN. Bioethanol production from fermentable sugar juice. The Scientific World J 2014;1-11.

[2] Thailand Department of Alternative Energy Development and Efficiency (DEDE). Ministry of Energy. The renewable and alternative energy development plan for 25 percent in 10 years (AEDP 2012–2021).

[3] Silalertruksa T, Pongpat P, Gheewala SH. Sustainability assessment of sugarcane biorefinery and molasses ethanol production in Thailand using eco-efficiency indicator. Applied Energy 2015;160:603-609.

[4] Sorapipatana C, Yoosin S. Life cycle cost of ethanol production from cassava in Thailand. Renewable and Sustainable Energy Reviews 2011;15:1343-1349.

[5] Thai Ethanol Manufacturing Association [Internet]. [cited 2016 Sep 20]. Available from: http://www.thai-ethanol.com/

[6] Trend of New York raw sugar price (2003-2014) [Internet]. [cited 2016 Sep 20]. Available from: http://www.areteonline.net/ aretepro/ tag/raw-sugar-fob-new-york/

[7] Thailand Department of Alternative Energy Development and Efficiency (DEDE), Ministry of Energy, Course of ethanol production from molasses and biogas production from ethanol plant, 2015.

[8] Shah SF. Enhanced production of ethanol from sugar cane molasses through thermotolerant Saccharomyces Cerevisiae cell [PhD Thesis]. Jamshoro, Pakistan: Mehran University of Engineering and Technology; 2010.

[9] Dubok C, Heung SS, Yu LP, Wu Y, Hoon C. Sugar production from raw seaweed using the enzyme method. J of Industrial and Engineering Chemistry 2009;15:12-15.

[10] Osman ME, Khattab OH, Hammad IA, El-Hussieny NI. Optimization of bio-fuel production by Saccharomyces cerevisiae isolated from sugar cane bagasse. J American Sci 2011;7:485-492.

[11] Chooyok P, Pumijumnog N, Ussawarujikulchai A. The water footprint assessment of ethanol production from molasses in Kanchanaburi and Supanburi Province of Thailand. APCBEE Procedia 2013;5:283-287.

[12] Report of ethanol price in the 4th quarter 2016, Bank of Thailand [Internet]. [cited 2017 Mar 15]. Available from: https://www.bot.or.th/Thai/MonetaryPolicy/NorthEastern/Doclib_Commodity Quater/Q1_59_Ethanol.pdf

[13] Thailand Sugarcane Breeding Center, Ministry of Industry [Internet]. [cited 2017 Mar 15]. Available from: http://www.ocsb.go.th/th/home/index.php

[14] Gnansounou E. Refining sweet sorghum to ethanol and sugar: economic trade-offs in the context of North China. Bioresource Tech 2005;96:985-1002.