The Journal of Applied Science **Vol. 16 Special issue: 132-140 [2017]**
วารสารวิทยาศาสตร์ประยุกต์ doi: 10.14416/j.appsci.2017.10.S20
ISSN 1513-7805 Printed in Thailand

Research Article

Nipa sap fermentation without yeast addition

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Abstract

Bioethanol production from nipa sap was studied in this work. Nipa sap is a source of sugars, minerals, yeasts and nutrients which can be suitably used as a raw material for ethanol fermemtation. The nipa sap fermentation was carried out sufficiently using the native yeast in the sap without external yeast addition and nutrient supplementation. Firstly, the sap was pretreated with pH 4.9 at 36 ºC for 25 min before fermentation. Response Surface Methodology (RSM) was employed to design minimally experimental conditions of the fermentation and to optimize the conditions. The studied variables were initial total sugar concentration (10–30 %w/w), initial pH (4.5–6.5), temperature (28–40 ºC) and time (10–144 hours). The optimal condition using 22.4%w/w initial total sugar with pH 4.5 at 29ºC for 77 h could achieve 91% ethanol yield with 112.1 g/L ethanol concentration.

Keywords: nipa sap, bioethanol, fermentation, native yeast

Introduction

Renewable energy, like biofuel, is the promising energy to reduce both consumption of petroleum-based fuel and greenhouse gas emission (Lang et al., 2001). Bioethanol, liquid biofuel, having some advantageous properties over gasoline such as higher octane number and flame speed (Balat, 2007), can be used directly or blended with gasoline (gasohol) and with diesel (diesohol) as transprotation fuel.

However, sustainability and economic viability for bioethanol production are considerable. In current, main feedstocks for the commercial bioethanol production are starch and juice or molasses (Wilkie et al., 2000; Mojović et al., 2006; Balat & Balat, 2009). Although lignocellulosic biomass or agricultural residue is the cheaper feedstock, bioethanol technology from the lignocellulosic biomass is confined to the laboratory. The sugar-based feedstock, juice or molasses, containing readily available fermentable sugar is easier than starch and lignocellulose to be used for producing ethanol. It can be carried out directly without costly steps including pretreatment and/or hydrolysis (Luo et al., 2014; Abdullah et al., 2015; Germec et al., 2015; Gumienna et al., 2016). In Thailand, there is only sugarcane giving juice and molasses that are used as the sugar feedstock. Therefore, it is needful to increase alternative feedstocks for the sustainability of this biofuel.

Palms can be alternative for the sugar feedstock, for instance coconut palm (*Cocos nufifera*), sugar palm (*Arenga pinnata*), palmyra palm (*Borssus flabellifer*) and nipa palm (*Nypa*

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fructicans), providing sap or sugar-rich juice. Especially, nipa palm which is a conservative plant in the projects initiated by Her Royal Highness Princess Maha Chaki Sirindhorn, is distributed all over Asia and Oceania (Pehutanan, 2009). The palm tree can be useful for coastal rehabilitation and land restoration of abandoned shrimp ponds.

Nipa sap is an attractive feedstock due that it has high content of fermentable sugar (sucrose, glucose and fructose) and collected easily causing no waste and no effect on the palm growth. Furthermore, the nipa palm grows in all areas naturally without the requirement for fertilizers, insecticides or electricity (Tumanaidu et al., 2013). It can provide, therefore, both the sap and good ecology.

However, there are several organisms namely acid tolerant bacteria, molds and yeasts in the sap due to its rich sugar minerals and nutrients (Tamunaidu et al., 2013). These organisms cause easily the sap becomes rancid and sugar in the sap is decomposed. These problems have been solved by traditional thermal process which wastes energy, loses nutrients and kills useful organisms like yeasts.

Hence, this study was to appreciate the full potential of the nipa sap which may be an efficient alternative feedstock for bioethanol production with utilizing all contents in the sap. This work focused on investigating the environmental factors on the organisms which could support the function of the native yeasts for the sufficient ethanol production without external yeast addition and nutrient supplementation. This process could reduce both the problems (spoilage and decomposition) and the production cost.

Materials and methods

Materials

Nipa sap was obtained from the Chan Tarang Sri planation located in Pak Phanang, Nakhon Si Thammarat province, Thailand. The fresh sap collected early in the morning (before 7.00 a.m.) was stored in screw-capped bottles instantly at 4°C untill use.

Nipa Sap Pretreatment

The fresh sap was filtered through a fabric filter to remove solid impurities. The filtered sap was adjusted to a pH of 4.9 with sulfuric acid solution and then heated using an oil bath at 36°C for 25 min (Based on our previous work).

Fermentation without yeast addition

The pretreated sap was cooled down to ambient temperature and diluted with deionized water to get substrates containing total sugar with different concentrations. The pH of substrate was adjusted to a designed value. The fermentation was proceeded with 100 g working volume in 250 mL air-locked flasks without yeast addition and nutrient supplementation. The flasks were placed in an incubator shaker (LabTech, LSI-3016A, South Korea) which was set a shaking speed at 80 rpm at an assigned temperature. Samples were collected at various times for the analysis of ethanol concentration.

Design experimental conditions by Response Surface Methodology (RSM)

The effect of factors were investigated using RSM. The fermentations were proceeded with initial total sugar concentration in the range 10-30 %w/w (g of total sugar in 100g of substrate or 106.3-318.9 g/L: g of total sugar in L of substrate), initial pH in the range 4.5-6.5, temperature in the range 28-40°C and time in the range 10-144 h. These variable ranges of the

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initial sugar concentration, pH, temperature and time were in the optimal ranges for the growth and function of yeasts (Charoenchai et al., 1998; Le & Le, 2014). A central composite design (CCD) was used with these variables, providing 27 total experiments (24 non-repeated cases and 3 repilcates of the center point) as shown in Table 1.

Table 1. Experimental conditions and the effects of process variables on the ethanol concentration and yield.

No	Process variables				Ethanol Concentratiom (g/L)		Ethanol yield (%)	
	S (%w/w)	pH	T (ºC)	t (h)	Experimental	Predicted	Experimental	Predicted
1	10	5.5	34	77	13.6	21.8	24.3	32.4
2	15	5	31	44	40.5	29.8	48.1	38.5
3	15	6	37	44	21.9	18.6	26.0	21.6
4	15	5	37	111	25.4	22.2	30.1	27.4
5	15	6	37	111	24.7	24.9	29.3	28.8
6	15	6	31	111	42.3	37.7	50.3	44.7
7	15	6	31	44	25.5	20.3	30.2	27.8
8	15	5	37	44	22.7	24.9	27.0	28.1
9	15	5	31	111	44.4	38.3	52.7	47.4
10	20	4.5	34	77	38.3	47.9	34.1	42.4
11	20	6.5	34	77	27.2	32.1	24.2	29.0
12	20	5.5	34	77	26.2	25.1	23.3	22.3
13	20	5.5	34	10	26.5	35.5	23.6	30.8
14	20	5.5	40	77	28.0	24.0	24.9	23.3
15	20	5.5	34	77	24.6	25.1	21.9	22.3
16	20	5.5	34	77	24.6	25.1	21.9	22.3
17	20	5.5	28	77	63.8	82.3	56.7	71.5
18	20	5.5	34	144	34.6	40.2	30.8	36.7
19	25	5	37	44	64.6	58.5	45.8	41.5
20	25	6	31	111	98.9	92.9	70.2	65.6
21	25	6	37	44	40.9	43.2	29.0	30.9
22	25	6	31	44	93.1	85.5	66.0	59.0
23	25	6	37	111	39.5	39.5	28.0	27.8
24	25	5	31	111	109.7	102.4	77.9	72.5
25	25	5	37	111	44.3	45.7	31.5	30.5
26	25	5	31	44	108.1	104.1	76.7	73.8
27	30	5.5	34	77	104.2	110.5	61.6	66.7

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Analytical methods

The sample was filtered through a syringe filter to obtain a clear liquid before analysis. Reducing sugar and total sugar concentrations were estimated by the dinitrosalicylic acid (DNS) method (Miller, 1959) and the modified phenol sulfuric method (Dubois et al.,1956), respectively, using a UV-Visible spectrophotometer (UV, HP 8453 with Chem Station software).

Ethanol concentration was determined using gas chromatography (GC6890 flame ionization detector, Hewlett Packard, USA). The temperatures of oven, detector and injector were kept at 85ºC, 150ºC and 230ºC, respectively. Nitrogen as carrier gas was fed at 25 mL/min while hydrogen and air as the combustion gas were 44.6 and 300 mL/min, respectively.
The theoretical ethanol yield was calculated as follow:

Ethanol yield (%) = { ethanol obtained in fermantation (g/L) /
[(0.511 x reducing sugar at the begining (g/L)) +
(0.538 x non-reducing sugar at the begining(g/L))] } x 100% (1)

where 0.511 and 0.538 are the conversion factors from reducing sugar and non-reducing (total sugar - reducing sugar) to ethanol, respectively.

Results and discussion

Nipa sap compositions

The raw sap used in this work composed 266.3 g/L initial concentration of total sugar (sum of reducing sugar and non-reducing sugar) having 36.1 g/L of initial reducing sugar and 7.0×10^4 cfu/mL initial native yeast. Both the reducing sugar (i.e. glucose and fructose) and the non-reducing sugar (i.e. sucrose) are fermentable sugar which can be converted into ethanol by the yeast.

The collection of the fresh sap in the morning and immediate pretreatment can reduce the spoilage and decomposition of the sap. This pretreatment, not only can increase sugar contents in the sap, but also can reduce the energy consumption and total production cost. The pretreatment at low temperatures which can support the yeast function (Underkofler et al., 1958) is better than at the high temperature in the traditional way. This was assured from the sugar and yeast cell contents were increased after the pretreatment which the pretreated sap contained 90.0 g/L reducing sugar, 313.9 g/L total sugar and 3.3×10^5 cfu/mL living yeast.

Effect of fermentation on ethanol concentration

Table 1. shows the experimental and predicted ethanol concentration results with the fitted regression model ($R^2 = 0.951$) which the analysis of varivance (ANOVA) is shown in Table 2.

The best fit of the ethanol concentration model with a second order polynomial is presented by Equation (2).

$$\text{Ethanol concentration (g/L)} = 1177.8 + 17.13S - 182.61pH - 45.11T + 0.0982t + 0.41S^2 + 14.9pH^2 + 0.779T^2 + 0.0028t^2 - 0.895S\times pH - 0.677S\times T - 0.01507S\times t + 0.583pH\times T + 0.135pH\times t - 0.02764T\times t \quad (2)$$

where S, pH, T and t are total sugar concentration (%w/w), pH value, temperature (ºC) and time (h), respectively. S^2, pH^2, T^2 and t^2 are the quadratic terms and S×pH, S×T, S×t, pH×T, pH×t and T×t are interactions.

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From *P*-value (Table 2.) implied that the linear terms T, the quadratic terms S^2, T^2 and the interaction term S×T are significant with $P < 0.05$.

Table 2. Analysis of variance (ANOVA) of the models.

Terms	Ethanol concentration		Ethanol yield	
	Coefficient	*P*-value	Coefficient	*P*-value
intercept	1177.8	0.05914	1292.0	0.01947
S	17.13	0.06501	6.700	0.368
pH	-182.61	0.122	-178.60	0.07916
T	-45.11	0.03165	-46.03	0.01273
t	0.09822	0.937	0.125	0.906
S^2	0.410	0.000371	0.272	0.00242
pH^2	14.90	0.101	13.41	0.08400
T^2	0.779	0.00586	0.697	0.00419
t^2	0.00283	0.155	0.00255	0.133
S×pH	-0.895	0.374	-0.414	0.624
S×T	-0.677	0.00124	-0.365	0.02057
S×t	-0.01507	0.318	-0.01536	0.234
pH×T	0.538	0.745	0.692	0.623
pH×t	0.135	0.369	0.119	0.351
T×t	-0.02764	0.274	-0.02417	0.260
R^2	0.951		0.905	
Adj R^2	0.894		0.794	
F	16.74		8.168	
F Signif	0.00001		0.0004	
Std Error	9.686		8.217	

Figures 1(a)-1(f). show the effects on the ethanol concentration. The ethanol concentration increased with increasing initial total sugar concentration for all pH levels (Figure 1(a)). An optimal ethanol concentration (>75 g/L) could be reached with an initial total sugar concentration higher than 20 %w/w (Figure 1(b)). On the other hand, the fermentation should not proceed at temperature higher than 31ºC (Figures 1(b), 1(d) and 1(f)), while there was no improvement in ethanol concentration with time further 84 h. (Figures 1(c), 1(e) and 1(f)).

Effect of fermentation on ethanol yield

The theoretical ethanol yield results summarized in Table 1 were used to fit the quadratic polynomial model for the yield, Equation (3).

$$\text{Ethanol yield (\%)} = 1292 + 6.7S - 178.6pH - 46.03T + 0.125t + 0.272S^2 + 13.41pH^2 + 0.697T^2 + 0.00255t^2 - 0.413S\times pH - 0.365S\times T - 0.01536S\times t + 0.692pH\times T + 0.119pH\times t - 0.02417T\times t \quad (3)$$

where S, pH, T and t represent total sugar concentration (%w/w), pH value, temperature (ºC) and time (h), respectively.

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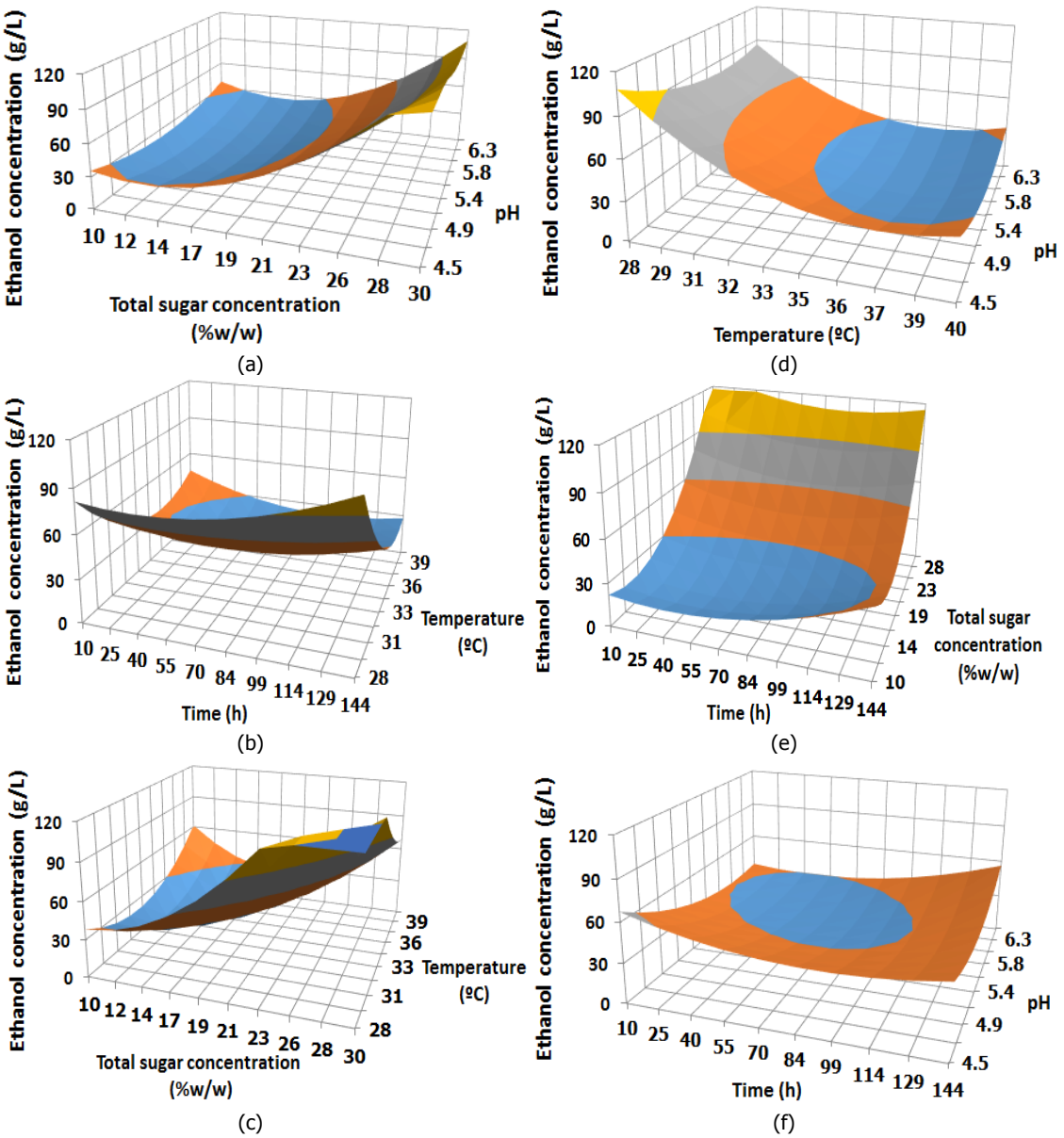

Figure 1. Ethanol concentration on the sap fermentation as a function of: (a) Total sugar concentration and pH at 34°C for 77 h, (b) Total sugar concentration and temperature with pH 5.5 for 77 h, (c) Total sugar concentration and time with pH 5.5 at 34°C, (d) Temperature and pH using 20 %w/w total sugar concentration for 77 h, (e) Time and pH using 20 %w/w total sugar concentration at 34°C and (f) Time and temperature with pH 5.5 using 20 %w/w total sugar concentration.

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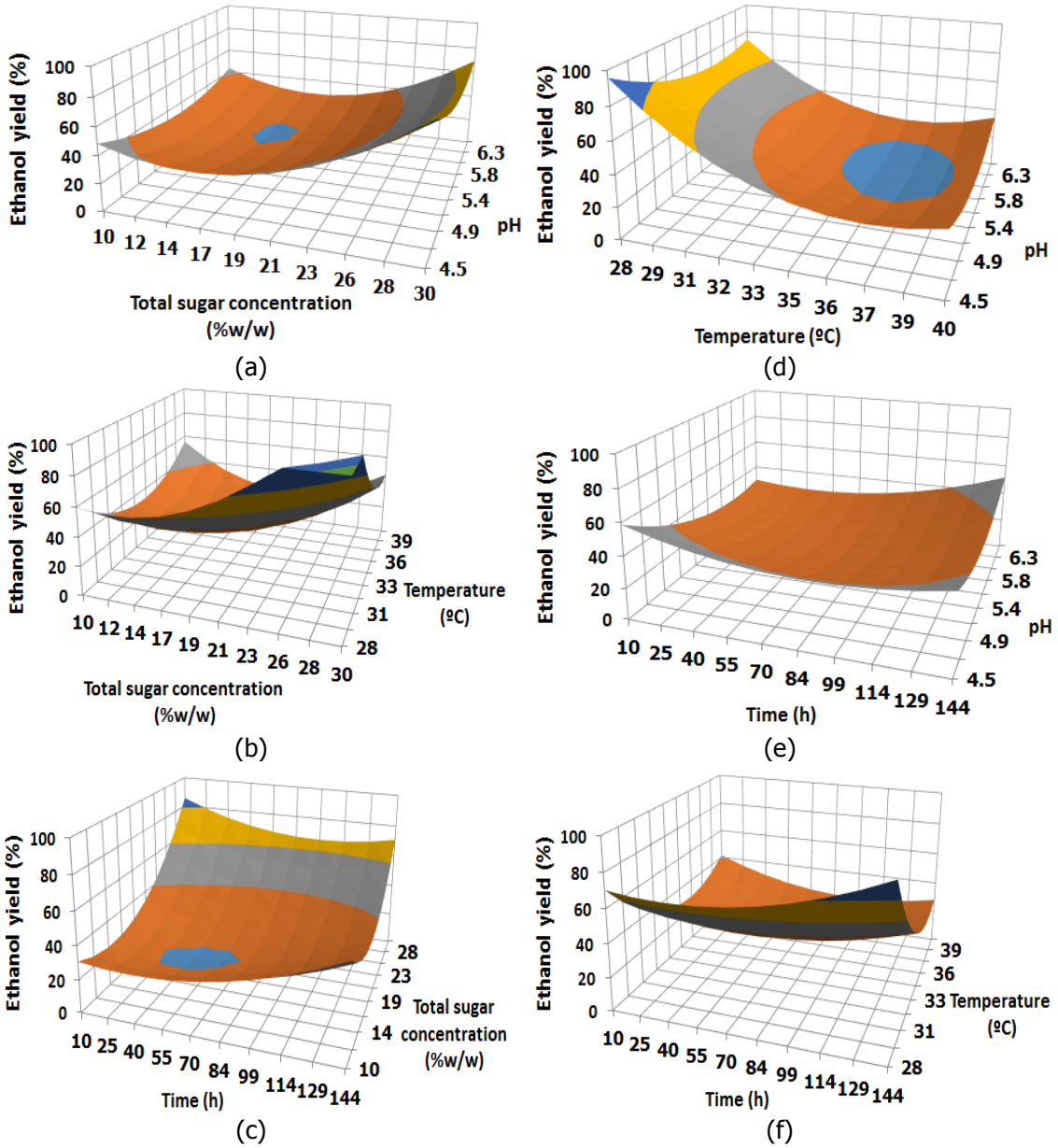

Figure 2. Ethanol yield on the sap fermentation as a function of: (a) Total sugar concentration and pH at 34°C for 77 h, (b) Total sugar concentration and temperature with pH 5.5 for 77 h, (c) Total sugar concentration and time with pH 5.5 at 34°C, (d) Temperature and pH using 20 %w/w total sugar concentration for 77 h, (e) Time and pH using 20 %w/w total sugar concentration at 34°C and (f) Time and temperature with pH 5.5 using 20 %w/w total sugar concentration.

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The initial total sugar concentration, pH, temperature and time effects on the ethanol yield are shown in Figures 2(a)-2(f). The results were the similar to the ethanol concentration response. The ethanol yield increased when total sugar concentration increased for all pH levels (Figure 2(a)) at temperatures below 31ºC (Figures 2(b), 2(d) and 2(f)), The result of pH effect implied that the studied pH range 4.5-6.5 was suitable for the yeast function (Le & Le, 2014). In addition, the fermentation time had the less influence on the yield (Figures 2(c), 2(e) and 2(f)). However, in order to achieve an optimal yield (>80%) the sap fermentation should be carried out using an initial total sugar concentration in the range 20-30 %w/w with an initial pH in the range of 4.5-4.7 at a temperature in the range of 28-31ºC for a sufficient time in the range of 70-80 h.

The ANOVA result for ethanol yield model is reported in Table 2. The main effects on the yield were individual and quadratic effects of temperature and its interaction with total sugar concentration. The quadratic effect of initial total sugar concentration was also significant for the fermentation.

Optimization of the nipa sap fermentation

The two responses indicate the rank order of the parameters affecting the fermentation without supplementation as: temperature > initial total sugar > initial pH > time.

From the model equations (2) and (3), the 138.3 g/L of the predicted optimum ethanol concentration would be reached using a total sugar concentration of 24.2 %w/w with a pH of 4.5 at a temperature of 29ºC for a fermentation time of 77 h. While the 100% ethanol yield would be obtained using 22.4 %w/w total sugar concentration with pH 4.5 at 29ºC for 77 h. The optimal concentration and yield were acquired under nearly similar conditions. Only the initial total sugar concentration was slightly different.

The two optimums are in the growth and metabolism ranges of the native yeast (Le & Le, 2014), therefore, the optimal fermentation which could be useful data for further development was 22.4%w/w initial total sugar with pH 4.5 at 29ºC for 77 h. Under this condition, the experimental ethanol yield was 91% with the experimental ethanol concentration of 112.1 g/L. This optimal yield achieved of 98.1% conversion ([g ethanol/g total sugars]/0.51) was higher than that of 70.8% achieved in prior work with raw sap collected from similar plantation site (Tumanaidu et al., 2013).

Conclusion

The results showed that nipa sap can be used as alternative feedstock for bioethanol production without supplementation. The nipa sap fermentation could be carried out sufficiently using the native yeast in the sap. These may reduce the total production cost and add value to nipa palm which is a conservative plant grown under a royal initiative project. In addition, this is the ethanol production along with maintaining good ecology.

Acknowledgement

This work was supported by the government budget of Prince of Songkla University, contract no. ENG5805895 and the Graduate School of Prince of Songkla University.

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