Production of Bioethanol from Palm Tree's Wastes by Enzymatic Hydrolysis and Fermentation

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Abstract: This work attempted to convert some of the wastes of palm tree (Phoenix dactylifera L.) into bioethanol, namely, leaflets, seeds and dates. The leaflets and seeds were ground and pretreated by three methods: direct fermentation, enzymatic hydrolysis (0.1 and 0.3) g/g and diluted alkaline (NaOH) followed by enzymatic hydrolysis. The dates were fermented directly by (Saccharomyces cerevisiae) with different yeast to dates ratios of (0.05, 0.1 and 0.25) g/g and at (25 and 35) ℃. The results showed that the highest bioethanol yields for leaflets and seeds were (13.3 ± 0.6) g and (15.5 ± 0.4) g, respectively, achieved by enzymatic hydrolysis 0.3 g/g for 48h, followed by fermentation by yeast to sample ratio of 0.1 g/g at 35 ℃. Whereas, the results of the dates experiments showed a shortest fermentation time of 7 h by yeast to dates ratio of 0.25 g/g at 35 ℃ at which the bioethanol production rate was 4.55 g/h. The maximum yield bioethanol of from dates was (31.9 ± 0.5) g. Correlations for the effects of temperature and yeast to dates ratio on the bioethanol production time were developed.

Keywords: Biomass; Waste; Palm Tree; Enzymatic Hydrolysis; Fermentation; Bioethanol.

1. Introduction

The need to use renewable and environmentally friendly sources of energy is increasing over time [1]. It was reported that Saudi Arabia had 31,234,155 palm trees in 2018 [2], which could be valuable resource of energy out of many other resources [3, 4]; as it was predicted that by 2034 the total potential bioenergy would be 8.0 million ton of oil equivalent [5]. This paper attempts to convert some of the palm tree's wastes (leaflets, seeds and dates) into biofuel (bioethanol) by using physical and chemical pretreatment methods proposed by researchers [6].

Numerous pretreatment methods of lignocellulosic biomass were found in literature. Zhu et al. [7] studied the effect of multiple concentrations of bisulfite and sulfuric acid on wood chips at 180 ℃ for 30 minutes. Bali et al. [8] studied the effect of various pretreatment alkalis on the glucose yield. They found that diluted NaOH at 120 ℃ for 1 hour caused the highest yield. Safari et al. [9] investigated the effect of diluted alkali pretreatments at three ranges of temperature, alkali concentration and retention time. They found 2% (w/v) of NaOH at 180 ℃ resulted in highest bioethanol yield. Raud et al. [10] explored the effect of three types explosive decompression gases at 175 ℃ and 30 bar. They found highest ethanol by using flue gas. Souto et al. [11] found that hydrothermal pretreatment by different concentrations of H_2SO_4 at 200 ℃ for 10 minutes resulted in highest ethanol yield. Borand et al. [12] found that pretreating by N_2 at 0.44 MPa and 190 ℃ for 22 seconds produced highest glucose yield. Sjulander et al. [13] reported that two steps pretreatment method by using nitrogen explosive decompression would recover high sugar yield.

Chemical analyses of date palm tree (Phoenix dactylifera L.) were carried out by many researchers. Siddeeg et al. [14] showed that 100g of Sukkary dates contain 78.32g of total sugars. Ismail et al. [15] found total sugars of 792.67 mg/g of Sukkary dates. Assirey [16] showed that the total sugars were 78.5g/100g of Sukkary dates. Aleid et al. [17] found that 100g of unpitted Sukkary dates contain 63.9g of total sugars. Nasser et al. [18] reported that 100g of Sukkary palm leaflets contain 47.14g, 16.13g and 36.73g of cellulose, hemicellulose and lignin, respectively. Whereas, date palm stones (seeds) contained 32.77g, 30.20g and 37.03g of cellulose, hemicellulose and lignin, respectively. Hindi et al. [19] reported that leaflets of palm date contained 36.44g of lignin per 100g.

2. Materials and Methods

2.1. Samples Preparation

Sukkary palm leaflets and seeds were collected and ground. The palm dates were dried in oven (202-1AB, Wincom, China) at 105 ℃ for 24 hours prior grinding. All samples were sieved by sieve shaker (Performer III SS-3, Gilson, USA). Most of leaflets and seeds passed through 600 µm sieve, whereas, most of the dates passed through 250 µm sieve. Figure 1 shows the samples in their original and ground forms.

Fig. 1. Some of Sukkary palm tree's wastes in their original and ground forms

2.2. Leaflets and Seeds

Three pretreatment methods were conducted for both leaflets and seeds samples. The first method was to ferment 100g of both leaflets and seeds by 10g of bread yeast (saccharomyces cerevisiae) in 400g of distilled water at 35 ℃ for 24 hours.

The second pretreatment method was simultaneous saccharification and fermentation. An enzymatic hydrolysis was conducted by using cellulase enzymes (Acozyme Cell, Acoma International, India). 100g of both leaflets and seeds were hydrolyzed by (10 and 30) g of cellulase enzyme in 400g of distilled water. They were kept at 50 ℃ for 48 hours. The oven's temperature was set to 35 ℃, then 10g of yeast were added to the samples and monitored for 24 hours. The pH measurements before the experiments for leaflets and seeds were approximately 5.8 and 6.6, respectively.

The third pretreatment method was to use NaOH as an alkaline material. 8g of NaOH were dissolved in 400g of distilled water, which made the pH raises to approximately 10.7. 100g of both leaflets and seeds were added to each solution. They were kept in oven at 50 ℃ for 3 hours. The pH of the solutions was decreased to approximately 5 by adding H_2SO_4. 30g of cellulase enzyme were added (10 and 30) g to both of the solutions. They were kept at 50 ℃ for 48 hours. The oven's temperature was set to 35 ℃, then 10g of yeast were added to the samples and monitored for 24 hours. For all previous methods, three replicates were used as shown in Figure 2.

Fig. 2. Enzymatic hydrolysis of palm leaflets and seeds

2.3. Dates

Experiments on dates powder were conducted at 25℃ and 35℃ with three ratios of yeast to sample (0.05, 0.10, 0.25) g/g. 100g of dried Sukkary dates powder were added to 400g of distilled water. They were kept in the oven for approximately 1 hour to reach the ambient temperature before the yeast was added. The glucose fermentation can be expressed as in Equation 1 [20]:

$$C_6H_{12}O_6 + \text{Yeast} \rightarrow 2C_2H_5OH + 2CO_2 + \text{Heat} \quad (1)$$

3. Results and Discussion

3.1. Leaflets and Seeds

For 24 hours, the average amounts of bioethanol yielded for different pretreatments of 100g of leaflets and seeds are shown in Table 1. For all pretreatments the distilled water was 400g.

Table 1. Average bioethanol yielded from leaflets and seeds after 24h of fermentation

Pretreatments	Leaflets	Seeds
None	(1.7 ± 0.3) g	(5.4 ± 0.3) g
Cellulase (10g)	(8.0 ± 1.1) g	(9.5 ± 0.5) g
Cellulase (30g)	(13.3 ± 0.6) g	(15.5 ± 0.4) g
NaOH (8g) + H_2SO_4 + Cellulase (10g)	(6.4 ± 0.4) g	(8.2 ± 0.3) g
NaOH (8g) + H_2SO_4 + Cellulase (30g)	(12.3 ± 0.5) g	(13.9 ± 0.2) g

The following Figures 3 and 4 shows the average cumulative bioethanol produced. Each line represents an average of three replicates. Generally, it can be noticed that the enzymatic hydrolysis by cellulase enzyme without pretreating with NaOH resulted in high bioethanol production for both palm leaflets and seeds. However, Pretreating the samples by NaOH followed by enzymatic hydrolysis would give better results than direct fermentation of samples without pretreatment. The bioethanol production was monitored by weighing the samples frequently during the reactions.

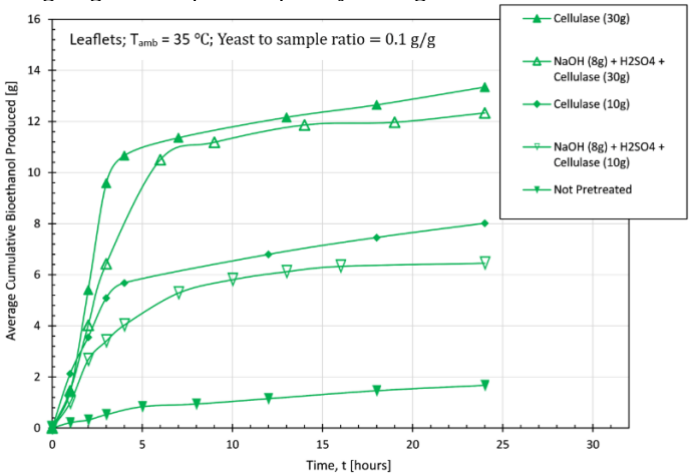

Fig. 3. Average cumulative bioethanol produced during 24 hours from 100g of Sukkary palm leaflets

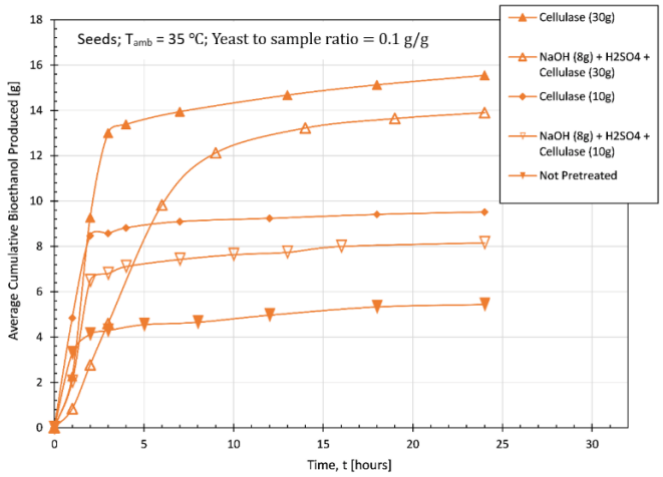

Fig. 4. Average cumulative bioethanol produced during 24 hours from 100g of Sukkary palm seeds

The curves in the previous two figures show that the rates of fermentation were high in the first two to eight hours (depending on the curve). Then, these rates slow down. Based on that, it could be said that most of the reaction was done within two to eight hours when the amount of yeast to sample was 0.1 g/g at 35°C. Also, the testing period (24 hours) was more than sufficient to ensure completeness of the fermentation. However, it was noted that some of the curves did not completely flatten, and this could be explained by the fact that the cellulase enzymes still breaking down complex sugars even after 48 hours have passed, and even with the presence of the fermentation reaction. Moreover, it could be seen that using H_2SO_4 as a neutralizing agent for NaOH had negative effect in fermentation process comparing to cellulase enzyme only. That effect was explained by P. Harmsen et. al [6], as they stated that the acid had a contribution in the formation of furfurals which are strong inhibitors to fermentation.

3.2. Dates

The following Table 2 summarizes the fermentation experiments of dried powder Sukkary palm dates for different treatments. The table also shows the number of hours at which the fermentation was assumed to be completed.

Table 2. Average bioethanol yielded from Sukkary palm dates.

Amount of Yeast (Per 100g of dates)	Temperature	
	25 °C	**35** °C
5 g	(31.5 ± 0.7) g ; 31 h	(31.5 ± 0.7) g ; 14 h
10 g	(31.9 ± 0.5) g ; 24 h	(31.3 ± 0.3) g ; 10.5 h
25 g	(31.0 ± 0.6) g ; 16 h	(31.7 ± 0.2) g ; 7 h

The previous data were plotted in detail in Figure 5. However, the production rates of bioethanol at 35 °C and 25 °C are shown in Figure 6. It was found that altering the yeast to dates ratio is linearly affecting the average production rate of bioethanol. Similar result was found in previous work at 30 °C for different yeast to dates ratios [21]. Also, similar result was found by another group for different feedstock [22].

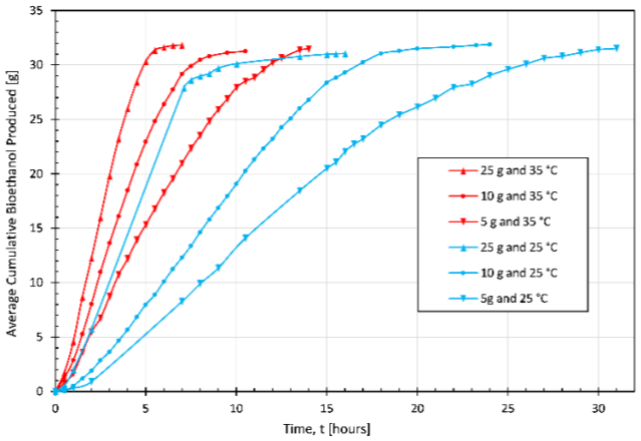

Fig. 5. Effect of temperature and amount of yeast on the average production time of bioethanol from 100g of dried Sukkary dates

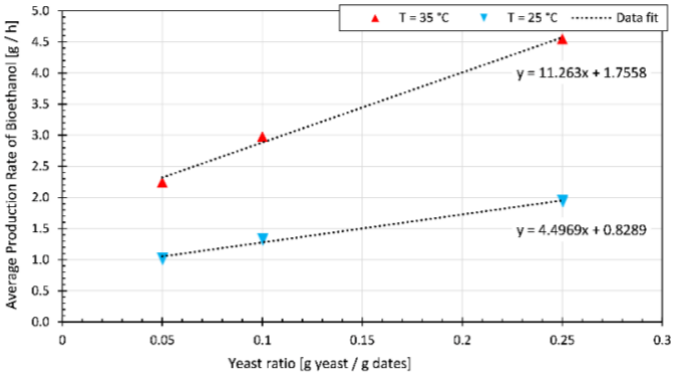

Fig. 6. Effect of temperature and yeast to sample ratio on the average production rate of bioethanol at 25 °C and 35 °C

A. Thygesen et. al [22] found that temperatures less than 40 °C were better than those of larger than 40 °C, in terms of yeast survival. They also found that the fermentation rate at 40 °C was similar to that of 25 °C. In this paper, 35 °C was chosen as an attempt to find the temperature at which the fermentation rate could be maximum. Whereas, 25 °C was selected as it is the commonly used temperature for conducting standard experiments. The previous data were processed in Design Expert software. ANOVA showed that the statistical model was significant. Data in Figures 5 and 6 could be represented by response surfaces that shown in Figures 7 and 8.

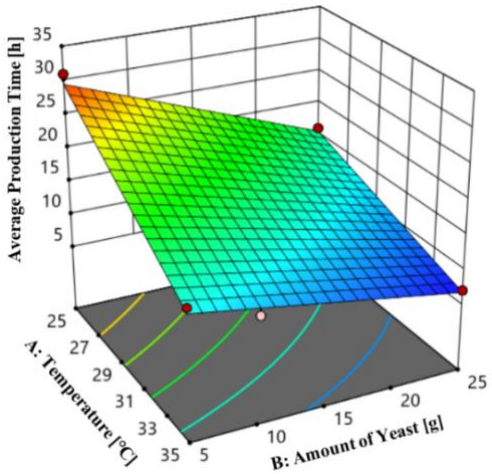

Fig. 7. Response surface of bioethanol average production time for 100g of Sukkary dates

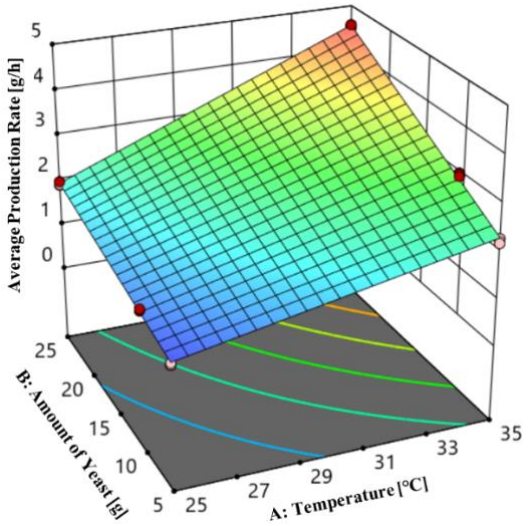

Fig. 8. Response surface of bioethanol average production rate for 100g of Sukkary dates

The previous data shown in Figures 7 and 8 were processed in Design Expert software. Analysis of variance showed that the amount of yeast, temperature and their interaction had significant influence on the average production time of bioethanol. The following Table 3 shows the detailed ANOVA for two factors interactions for bioethanol production time.

The following two correlations were obtained based on previous response surfaces. They are valid in the ranges (25 to 35) ℃ and (0.05 to 0.25) g yeast/g Sukkary dates.

$$\text{Production Time [h]} = 78.48 - 1.82\ T - 164.23\ Y + 3.77\ T\ Y \quad (2)$$

$$\text{Production Rate [g/h]} = -1.48 + 0.09\ T - 12.54\ Y + 0.68\ T\ Y \quad (3)$$

4. Conclusion

In this paper, some of the wastes of palm tree were converted into bioethanol. Namely, leaflets, seeds and dates. All samples were ground in fine sizes. The major findings in were: i) pretreating the ground leaflets and seeds by enzymatic hydrolysis using cellulase yielded higher bioethanol than direct fermentation or using NaOH for 3 hours at 50 ℃ followed by H_2SO_4 and cellulase. ii) The maximum bioethanol yields from leaflets and seeds were (13.3 ± 0.5) g and (15.5 ± 0.3) g, respectively, achieved by cellulase to sample ratio of 0.3 g/g iii) The shortest fermentation time for Sukkary palm dates was 7 hours by yeast to dates ratio 0.25 g/g at 35 ℃. iv) Correlations for bioethanol production from Sukkary dates were developed as two factor interaction. They are valid in the studied ranges.

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Table 3. Analysis of variance (ANOVA) for two factors interaction (Bioethanol production time)

Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
Model	1166.47	3	388.82	216.41	< 0.0001	Significant
A-Temperature	681.26	1	681.26	379.17	< 0.0001	-
B-Amount of Yeast	340.17	1	340.17	189.33	< 0.0001	-
AB	46.17	1	46.17	25.7	0.0002	-
Residual	25.15	14	1.8	-	-	-
Lack of Fit	25.15	2	12.58	-	-	-
Pure Error	0	12	0	-	-	-
Cor Total	1191.63	17	-	-	-	-

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