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The Study of Suitability of Alkaline Treatment for Lignin Reduction in Banana Stem and Peel Using Response Surface Methodology

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Abstract. The use of banana by-products as ruminant feed has been applied in the country with high banana production. Yet, there is still a lack of research conducted to identify the digestibility of banana plantain in ruminants as high lignin content led to poor degradability by rumen micro-organism. This study investigated the suitability of lignin reduction in banana stem (BS) and peel (BP) using alkaline pre-treatment. Three parameters were tested in this study. The parameters were the concentration of sodium hydroxide (1m, 5.5m, 10m), contact time (1 hour, 6.5 hours, 12 hours) and weight of the sample (0.5g, 2.75g, 5g). The correlation coefficients for both responses were performed by using a central composite design (CCD). The correlation coefficient values for banana stem and banana peel were 0.8632 and 0.4124, respectively. The optimum lignin removal condition for banana stem predicted by response surface methodology (RSM) was found at 6% with 9.76h, 7.92m and 4.98g, and banana peel at 10.09% with 1h, 10m and 0.5g. It demonstrates that alkaline pre-treatment using sodium hydroxide can effectively remove the lignin content in banana stem and banana peel. The effectiveness, however, is also affected by the sodium hydroxide concentration and contact time, respectively, for banana stem and banana peel due to their difference in structure. In conclusion, this study found that alkaline pre-treatment reduces the lignin content in banana stems and banana peels.

INTRODUCTION

Feed insecurity is one of the critical problems for the farmers in the livestock industry. In Malaysia, almost all the raw material used as the feedstuff was imported, and only a few of the raw materials produced locally from agricultural by-products such as molasses from sugar cane, broken rice, rice husk, sago, tapioca [1] and palm kernel extract (PKE) [2]. Some farmers used imported pellets and mixed them with the other feed material [3] and different types of grass to reduce the pellet cost [4]. Recently, the agricultural by-products from fruits and vegetables have been used as the alternative materials to feed the livestock animals [5]. The initiative is to reuse the agricultural residue while reducing the issues on social and environmental [6,7]. The over-production of food products worldwide led to the increase of

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food residue and wastes [8]. The effect from implantation of applying by-products from the agro-industrial field as a nutrient to the livestock animals, it will increase the income of the industry itself as by implying the agriculture residue as feed it will improve the quality of the food material and it is very economical to the farmers [9].

Banana is well known for its high nutrition content of potassium and sodium [10]. In Asia, the biggest exporter of bananas is the Philippines, with a total export of 2.7 million tonnes, increasing by 71,000 in 2012 and making it the second-largest exporter globally after Ecuador [11]. The banana plant is well known as the largest herbaceous plant that only bears fruits once in its life cycle. It is from the family Musaceae and scientifically called *Musa spp*. In Malaysia, the most common banana species is *Musa paradisiaca l.*, obtained from the cross of breed *Musa acuminata* and *Musa balbisiana,* and is also known as plantain or cooking banana. As the most produced fruits globally, bananas statistically in 1988 global production of bananas and its plantain was 41.9 million tonnes and 24.0 million metric tons. Respectively, in which if presume that up to 40% of the total was turned down during the quality check for export; it has the highest potential to be used as livestock feeding materials [12].

Lignin in bananas acts as a barrier to the animal digestion system due to its inability to digest cellulose, and hemicellulose needs to be reduced before feeding to the ruminant [13]. Feed quality is determined by the suitable feedstuffs that have high nutrient content for the animals. The banana residue still can be used with the involvement of technology such as pre-treatment to reduce the component and increase the digestibility of the banana plantain when fed the ruminant. Chemical treatment is much easier and effective than biological and physical treatment that needs more energy and is more time-consuming. This study focuses on optimising chemical treatment by using Sodium Hydroxide applied to the banana peel and stem to remove the lignin content in the sample by enhancing the optimum variable condition for lignin removal by using Response Surface Methodology (RSM) [14, 15].

MATERIALS AND METHODS

Materials Used in This Study

Materials used in this study were banana stems and ripe banana peels. The stem was collected in Agrotechnology Park in UMK Jeli, Kelantan and was placed in the zip lock bag to retain its freshness. Meanwhile, the peels were collected from Universiti Malaysia Kelantan, Jeli, Kelantan and stored in the refrigerator to avoid over-ripening. The chemicals used in this study were sodium hydroxide (NaOH), hydrochloric acid (HCI) and distilled water. The equipment used in this study were analytical balance, beaker, Whatman Filter papers (250mm), filter funnel, micropipette and tips, beaker (250ml, 1L, 5L), conical flask (250ml), spatula, aluminium foil, dropper, gloves, oven, hot plate stirrer, pH meter, reagent bottle. Design-Expert Software Version 10.0 was used in this study.

Sample Preparation

Banana peels and stems were cleaned to remove dirt and cut into small pieces. Then, the samples were dried in the oven at 60ºC for 12 hours to remove the water content before it can be treated. The samples were weighed 0.5g, 2.75g and 5g.

Preparation for Alkaline Pre-treatment

NaOH tablets were used to obtain the 1M, 5.5M and 10M concentration for each experiment run. 5g, 22g and 20g of NaOH tables were dissolved with 100ml distilled water in three separate 250ml beakers. The solution was made before each experiment started. Weighted banana peels and banana stem were added into 1M, 5.5M and 10M NaOH following its suggested parameters. Black liquor was filtrated and acidified to 3.5 Ph. The product was then let dry overnight in the oven at 60ºC [16].

Experimental Design Using Response Surface Methodology

Response Surface Methodology (RSM) tool was used to determine the parameters that will influence the process of lignin removal. It provides the optimum sodium hydroxide (NaOH) concentration, duration of time, and weight of the pre-treatment process after adding an average value in the RSM in Design-Expert Software (Version 10.0). The

CCD was set at zero for both 'numeric factors' and 'categoric factors'. Since three parameters were studied in this study, the numeric factor is three—the information tabulated in Table 1.

TABLE 1. Experimental design using Central Composite Design (CCD)

Variable	Name	Units	Low level (-1)	High level (+1)
A	Concentration of NaOH	Molarity	1	10
B	Contact Time	hours	1	12
C	Weight	g	0.5	5

Next, the face-centred option chosen was to proceed with alpha, alpha equal to 1. Option in the face centred indicating the level of study are only three levels, minimum (low), medium (middle) and maximum (high). In this experiment, the lignin removal percentage of banana peel and banana stem was used as the response. From the design, 20 runs were generated for each sample (40 in total) in which each of the operating was different, as shown in table 2.

TABLE 2. The total experiment runs generated using the CCD model

Std Run	A: NaOH Dosage	B: Contact Time	C: Gram
1	1	1	0.5
2	1	12	5
3	5.5	6.5	2.75
4	10	12	0.5
5	5.5	6.5	2.75
6	5.5	6.5	0.5
7	1	12	0.5
8	1	6.5	2.75
9	5.5	6.5	5
10	5.5	6.5	2.75
11	5.5	12	2.75
12	10	1	0.5
13	10	6.5	2.75
14	1	1	5
15	5.5	6.5	2.75
16	10	1	5
17	10	12	5
18	5.5	6.5	2.75
19	5.5	1	2.75
20	5.5	6.5	2.75

RESULTS AND DISCUSSION

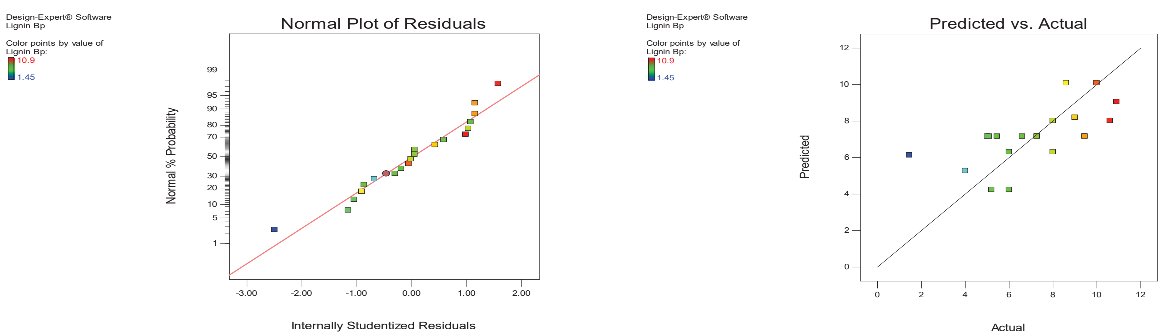
The correlation coefficient was used to measure the fit of the model, R^2, and the model's accuracy is considered high if the value of R^2 is closer to 1.00. Theoretically, if the R^2 value was proximally 1.00, this indicates the developed model is valid and able to forecast effective feedback [15]. In this experiment, the R^2 obtained is 0.8632, and it shows that the model for banana stem can explain 86% of the response variability.

Analysis of variance (ANOVA) was used in the study to justify the significance of the models; the F value is 7.01, and the p-value is 0.0027, which indicate that the model is significant, as shown in Table 3. In this study, the largest F-value is 10.29 for factor B (contact time). Thus it shows that contact time is the most critical factor in lignin removal in the banana stem. The highest percentage of lignin degraded from the lignin is 6%, with the optimum condition of 10M of sodium hydroxide concentration, 12 hours of contact time and 0.5 g of sample. The actual value is not much different from the predicted value, which is 6.6%. The data point lies reasonably closed to the linear line. This indicates that the residuals were small and distributed normally (Figure 1(a)). From the diagnostic plot (Fig. 1(b)), the residuals were distributed randomly but still relatively closed to the linear line indicated that the quadratic model was adequate to predict the response of lignin removal over the range of the parameters studied.

TABLE 3. ANOVA table for response surface quadratic model equation of banana stem

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	49.89	9	5.54	7.01	0.0027	significant
A-NaOH dosage	5.60	1	5.60	7.08	0.0239	
B-Contact Time	8.14	1	8.14	10.29	0.0094	
C-Gram	1.94	1	1.94	2.45	0.1487	
AB	0.98	1	0.98	1.24	0.2916	
AC	5.12	1	5.12	6.48	0.0291	
BC	0.080	1	0.080	0.10	0.7570	
A^2	4.12	1	4.12	5.22	0.0455	
B^2	6.99	1	6.99	8.84	0.0140	
C^2	27.04	1	27.04	34.20	0.0002	

A-NaOH concentration; B-Contact time; C-Weight of sample in gram



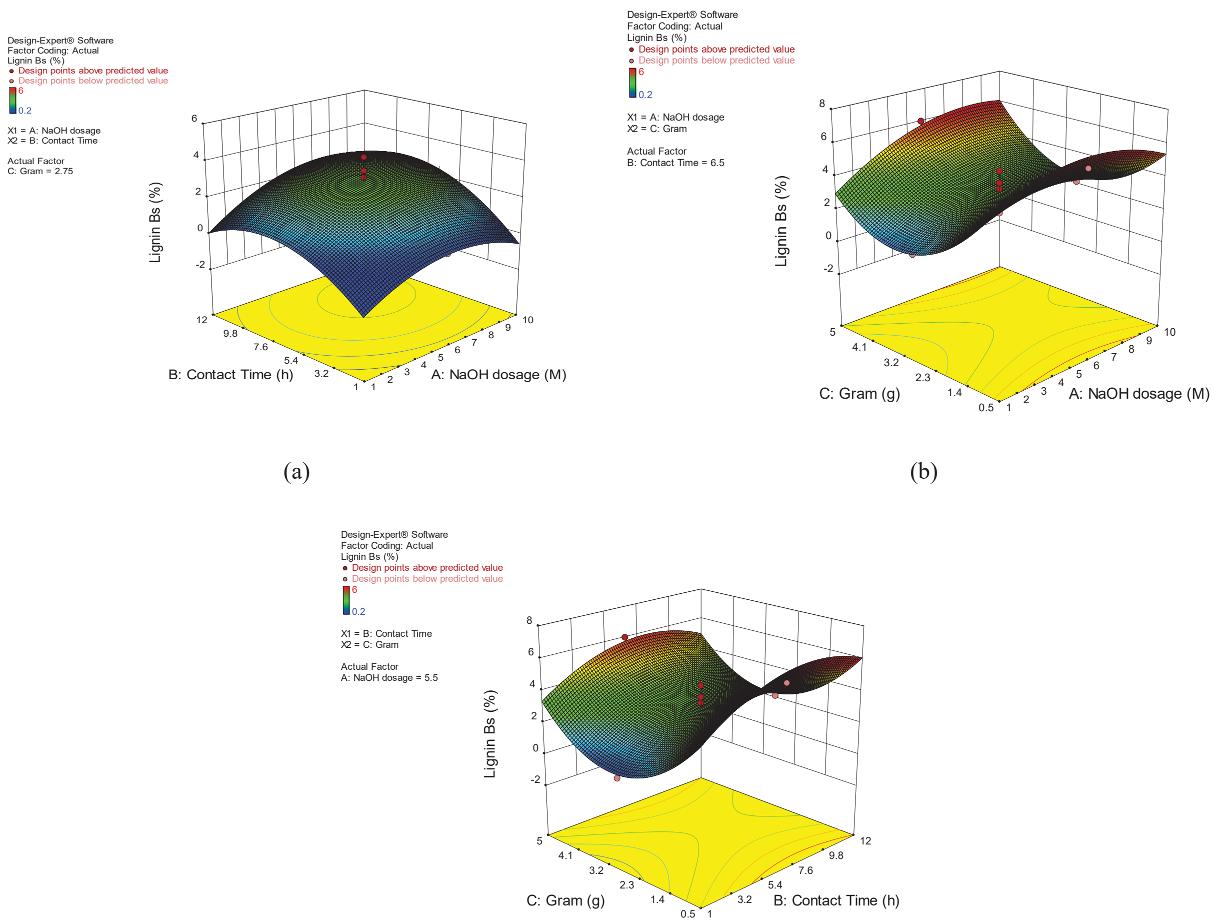
(a) (b)

FIGURE 1. (a) Normal probability plot of residuals for lignin removal in banana stem and (b) diagnostic plot for predicted values versus actual values of lignin removal in Banana stem

A three-dimensional (3D) response surface graph was obtained from the software to determine the optimum level of variables to achieve optimum lignin removal. The combination effect of adsorbent dosage and contact time on the percentage of lignin removal is shown in Fig. 2 (a). The weight of the sample at this combination effect is constant at 2.75g. The increases of concentration of NaOH from 1 to 6 molarity enhance lignin degradation and increasing the lignin degradation. This may cause by the complex structure of lignin that cannot be degraded by the NaOH treatment alone, as a combined pre-treatment is more effective than sole alkali pre-treatment on lignin removal [18]. The alkali solution had saturated the receptor and eventually became a constraint to the absorption of free chemical particles in the alkali solution, resulting in low lignin removal [19]. Time taken does not effectively affect the lignin removal as it is not the factor in destructing the bounded structure of lignin to release the lignin. The maximum percentage of lignin removal was 2.5% at 7.6 h and 6ph, with the constant weight of the sample at 2.75g. Suppose the sample weight exceeds 2.75 g and the lignin removal percentage does not increase. In that case, there are more unused adsorption sites in the total surface area of the sample than the adsorbate in NaOH solution [20].

Fig. 2 (b) shows the interaction between NaOH concentration and sample weight, and contact time is kept constant. The changing colour from green to red indicate the increase of lignin removal. The percentage of lignin removal increase as the concentration of NaOH increase. This may be contributed by the surface area expose of smaller size of samples to the NaOH is higher than when the weight sample more. The maximum removal of lignin is at 6% under the condition 7 mol at 2.3 g mass of sample with constant contact time at 6.5 h.

The combined effect of time taken and sample weight while keeping NaOH concentration constant (Fig. 2 (c)). As the time increases and at the low weight of the sample the lignin removal percentage is higher. The red zone shows the high percentage of lignin removal, which is at 6% when the contact time is 12 h, and the weight of the sample is 2.3g and contact pH at 5.5 M.



(a) (b)

(c)

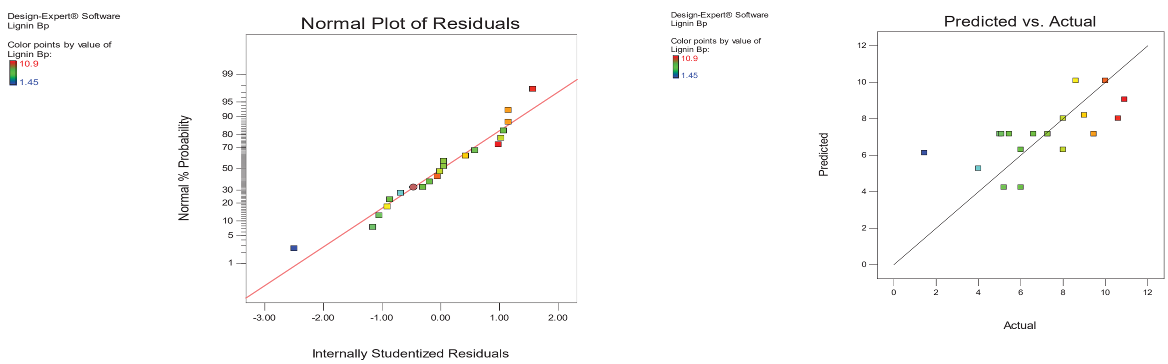
FIGURE 2. 3D response surface graph on the interaction between the effect of concentrations (M) and contact time (h) (a), the interaction between the effect of concentrations (M) and weight of the sample (g) (b) and graph on the interaction between the weight of the sample (g) and contact time (h)

A model of summary generated suggested that the linear model was the best that fit for the lignin removal in the banana peel. The R^2 obtained is 0.4124, of the response variability, can be explained by the model for the banana peel. The F value of 3.74 implies that the model is significant (table 4). Hence, there is only a 3.27% chance that an F-value this large could occur due to noise. Other than that, the p-value is 0.0327, which is greater than 0.0500 indicate the model terms are significant. In this study, factor A (NaOH dosage/concentration) is the most significant influence on the lignin removal in the banana peels, followed by factor B, contact time and factor C, gram or weight of the sample. The highest percentage of lignin removal in banana peel is 10.9%, with the optimum condition at 10M, 6.5hours and 2.75 grams of sample. The actual response in 9.06%, and it was slightly different from the actual value. Zahoor [21] mentioned that agitation speed has directly proportional to the removal process. This can be explained by high turbulence speed will lower the boundary layer of resistance around the sample and enhance the removal rate. In other words, agitation cause kinetic movement effect in NaOH solution and absorbent, which aids collision effect for better lignin removal rate [22]. Jayabal et al. [23] reported lower lignin level when increase alkali concentration. Nikzad et al. [24] mentioned that 30 minutes of contact time with NaOH solution allows highest lignin removal among 15-45 minutes of the test while Dong et al. [25] mentioned that among 20 minutes till 120 minutes test, 120 minutes showed the best lignin removal result. Wang et al. [26] fixed 24 hours in all the lignin removal experiments. This indicated various NaOH pre-treatment contact periods, but most of the articles reported better results in longer contact time.

TABLE 4. ANOVA table for response surface linear model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	46.43	3	15.48	3.74	0.0327	significant
A-NaOH dosage	35.72	1	35.72	8.64	0.0096	
B-Contact Time	10.71	1	10.71	2.59	0.1270	
C-Gram	1.421E-014	1	1.421E-014	3.437E-015	1.0000	

The data was used to plot a normal probability of residual, as shown in Fig. 3 (a). The figure shows that the residual lie reasonably close to the line, indicating that the residual was normally distributed. On the other hand, Fig. 3 (b) shows the relationship of actual versus the predicted value of the percentage of lignin removal in the banana peel.



(a) (b)

FIGURE 3. Normal probability plot of residuals for lignin removal in banana peel (a) and Diagnostic plot for predicted values versus actual values of lignin removal in banana peel (b).

CONCLUSION

The optimum lignin removal condition for banana stem was found at 6% with 9.76h, 7.92m and 4.98g, and banana peel at 10.09% with 1 h, 10m and 0.5g. Alkaline pre-treatment by using sodium hydroxide can effectively remove the lignin content in banana stem and banana peel. The effectiveness of lignin removal is also affected by the sodium hydroxide concentration and contact time, respectively, for banana stem and peel due to their difference in structure.

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