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The optimization of sodium hydroxide (NaOH) pre-treatment for reducing sugar production from rice husk using response surface methodology (RSM)

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ABSTRACT

Lignocellulosic biomass is an abundant renewable resource which amounts to 1.3 billion tonnes per year and can be used in a variety of sustainable applications. It is primarily made up of tightly bound cellulose, hemicellulose, and lignin. However, hemicellulose dissolution and delignification pose significant challenges to the utilisation of reducing sugar. To solve this issue, alkaline pretreatment was utilised. The aim of lignocellulosic biomass pretreatment is to break down the complex structure of the biomass and provide better access to the components that can be converted into useful reducing sugar. Response Surface Methodology (RSM) was used to identify the optimal values for the factors influencing the pretreatment. The optimisation parameters were sodium hydroxide concentration (1 – 4 % w/v), pretreatment time (15 – 60 minutes), and solid loading (6 – 16% w/v). Rice husk, which was used as biomass, was hydrolysed enzymatically to produce reduced sugar. The optimum conditions for producing the highest reducing sugar of 15.18 mg/mL from rice husk were 1.67% w/v sodium hydroxide pretreatment, 59.44 minutes of pretreatment time, and 7.67% w/v solid loading. As a result, less alkaline reagents can be used with a longer pretreatment time, thus lowering the cost of pretreatment. Therefore, this shows that RSM was one of the best methods to replace the conventional technique for optimising the parameters of rice husk alkaline pretreatment for reducing sugar production.

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1. INTRODUCTION

Nowadays, the rising occurrence of non-communicable diseases (NCDs), particularly hypertension and obesity, is regarded as a serious public health issue. Due to consumer demand for a diet that links health and nutrition, the functional food market is expanding to increase the proportion of nutritious ingredients added to food. In this line, consumers are looking for products with lower fat and salt content and functional food ingredients (prebiotics and probiotics) that aid in health care. As a result, developing clean and renewable energy to replace bio-based products and ingredients is urgently needed.

Because of its abundance and bio-renewable biomass on Earth, lignocellulosic biomass is gaining popularity among researchers. Previous research has shown that lignocellulosic biomass, such as rice husk, can be used as a raw material for reducing sugar production. The primary structural component of these plant cell walls is lignocellulose. The core of cell walls is made of cellulose, providing them with rigidity. Hemicelluloses serve as plant structural and reserve materials. Lignin is ultimately responsible for cell wall endurance because it envelops the microfibril core and stabilises and protects its structure [1]. The lignocellulose content is determined by its origin and source, growing method, and harvesting procedure.

Rice husk is a type of lignocellulosic agricultural biomass primarily derived from the rice milling and agriculture-based biomass industries. According to the USDA Foreign Agricultural Service (2017), Malaysia's rice production is expected to increase from 1.8 million tonnes in 2016 – 2017 to 0.02 million tonnes in 2017 – 2018. However, rice husks, a byproduct of the rice-milling industry, account for approximately 20% of rice weight and cause disposal issues when burned or disposed of as agricultural waste in landfills [2]. Thus, rice husk has evolved into a valuable biomass with the potential for long-term environmental expansion. The expansion of agricultural waste, such as rice husks, is being used to generate biogas and energy instead of being burned in an open field, which negatively impacts the environment and the population's health.

Pretreatment methods for extracting reducing sugar from rice husk include physical, chemical, and physico-chemical pretreatment [3]. Chemical treatment uses organic or inorganic agents to change the structure of lignocellulosic materials. In comparison to other chemical pretreatments, alkaline pretreatment uses less hazardous substances such as ammonia, sodium hydroxide, and lime. The reactivity of the residual polysaccharides is improved by alkaline pretreatment that removes lignin. Lignin is a large, complex molecule comprising phenolic monomers that are cross-linked polymers. In the main cell wall, it provides structural stability, impermeability, and antimicrobial resistance. The structure of lignin in biomass can be altered by decomposing the side chains of esters and glycosides, resulting in swelling, cellulose decrystallization, and hemicellulose solvation [4]. Sodium hydroxide, potassium hydroxide, calcium hydroxide, and ammonium salts are the most commonly used alkaline reagents. Because it is safe and inexpensive, sodium hydroxide is the most effective and widely used alkaline reagent among these hydroxyl derivatives [5].

Lignocellulose is a complex carbohydrate combination that requires an effective pretreatment to allow pathways for enzymes to generate fermentable sugars. Renewable lignocellulosic raw materials are low-cost feedstocks that promote industry sustainability. As a result, alkaline pretreatment is required to reduce sugar from lignocellulosic biomass [6]. To achieve a high reducing sugar conversion, the alkaline pretreatment must be optimised using the Response Surface Methodology (RSM) technique from Design Expert software to determine the optimal values for the pretreatment factors [7].

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2. METHODOLOGY

2.1 Preparation of biomass

The rice husk was soaked, washed and rinsed in tap water to remove dust and other pollutants. Then, it was dried in an oven at 110 °C for 12 hours or until the moisture content reached below 10%. The biomass was pulverised using a grinder (Miniature Grinder, Mill Powder Tech) and sieved using a mechanical sieve shaker (Analysette 3 Spartan) to achieve a consistent particle size of 250 µm. The screened rice husk was then stored in a sealed plastic bag and kept at room temperature until the next use. Otherwise specified, all compounds are of analytical grade.

2.2 Optimization by using response surface methodology (RSM)

The production of reducing sugar through alkaline pre-treatment was influenced by a number of parameters. RSM by a Design Expert was used to determine the optimal values for these parameters: concentration of sodium hydroxide (1 – 4% w/v), pre-treatment time (15 – 60 minutes) and solid loading (6 – 16% w/v) [8][9]. The pre-treatment temperature was set constant at 80˚C. Analysis, including mathematical and statistical analysis, was performed to determine the effects and interactions of the parameters. Three-dimensional surface plots will illustrate the influence of parameters on the response. RSM will optimise the parameters influencing the alkaline pre-treatment. Five points (-α, 1, 0, +1, +α) were assigned to each optimization step, with Central Composite Design used as the model.

2.3 Alkaline Pre-treatment

15 samples of rice husk were prepared based on the parameters set from RSM. The rice husk was weighed based on 15 sample sets, as referred to in RSM. Then, the rice husk samples were soaked in sodium hydroxide and NaOH at different pre-treatment time sets. A water bath was used to follow the constant pre-treatment temperature of 80 °C for 15 minutes. After that, the samples were rinsed using distilled water to remove the remaining alkali from the surface of the rice husk using a nylon cloth filter. The filtered samples were dried in an oven at 100 °C for 24 hours.

2.4 Enzymatic hydrolysis

The treated samples were hydrolysed in a 50 mM sodium citrate buffer at pH 4.8. The hydrolysed samples were then incubated at 50 °C for 15 minutes in the water bath. Approximately 90 U per gram of xylanase enzyme were added to each sample prepared. The hydrolysis was performed in an incubator shaker at 50 °C and 120 rpm for 24 hours. After hydrolysis, the samples were stored in the refrigerator at −20 °C for the next reducing sugar analysis.

2.5 Reducing sugar analysis by using DNS assay

The samples were centrifuged at 4800 rpm for 15 minutes using a centrifuge. The samples were then filtered using filter paper, and the supernatant was taken as the analysed sample. About 0.1 mL of rice husk sample and 0.9 mL of DNS reagent were added to the test tube. The samples were heated at 100 °C for 15 minutes to stop the enzymatic reaction. The samples were diluted with distilled water for 10 mL of total volume. It was then homogenised and read using a UV-VIS Spectrophotometer at an absorbance of 540 nm. A standard curve was also generated by plotting the optical density and known reducing sugar concentration. As a result, the concentration of reducing sugar that is known can be calculated. The xylose standard curve was used to illustrate the relationship.

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3. RESULTS AND DISCUSSION

3.1 RSM optimization for rice husk in alkaline pre-treatment

In this alkaline pre-treatment, 15 pre-treatment experiments with varying input conditions were carried out. Table 1 shows the DNS-determined coded values for the three independent variables and the dependent variable. Rice husk pre-treatment with the highest NaOH concentration (4% w/v) and solid loading (16% w/v) but the shortest pre-treatment time (15 minutes) compared to other experimental runs resulted in the highest sugar yield of 14.44 mg/mL. This suggests that a high concentration of NaOH is required to extract more reducing sugar from lignocellulosic biomass. The reducing sugar yield ranges from 8.28 to 8.71 mg/mL on average. This result was achieved with a NaOH concentration of 2.5% w/v. In comparison to other concentrations, the value of NaOH concentration was moderate. As a result, the optimisation of the experimental run from the RSM demonstrates that the conditions provided were logically acceptable for the production of reducing sugar.

Table 1. Central Composite Design experimental design of RSM with three independent variables and measured dependent variable, reducing sugar yield (mg/mL)

Experimental run no.	x_1 (% NaOH, w/v)	x_2 (time, min)	x_3 (% solid, w/v)	Reducing sugar yield (mg/mL)
1	2.5	37.5	16.0	7.94
2	2.5	37.5	11.0	8.28
3	2.5	37.5	11.0	8.28
4	2.5	37.5	11.0	8.28
5	4.0	15.0	16.0	14.44
6	2.5	37.5	11.0	8.28
7	4.0	37.5	11.0	3.74
8	1.0	60.0	16.0	9.51
9	2.5	37.5	11.0	8.28
10	2.5	60.0	11.0	8.31
11	2.5	15.0	11.0	8.71
12	4.0	60.0	6.0	5.35
13	2.5	37.5	6.0	10.39
14	1.0	37.5	11.0	10.42
15	1.0	15.0	6.0	3.47

3.2 Regression analysis and ANOVA

A quadratic model was used to describe the mathematical connection between the independent variables and the dependent variable. The regression model was obtained for both coded and real components, where a positive sign indicates synergistic impact and a negative sign indicates antagonistic influence [10]. Eq. (1) and Eq. (2) show the coded and actual equation of the regression analysis, respectively. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor, and the intercept is not at the centre of the design space [10].

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$$Total\ sugar\ concentration\ coded = 8.27 - 3.34A - 0.20B - 1.22C - 5.01AB + 0.5575AC - 5.04BC - 1.20A^2 + 0.2353B^2 + 0.8853C^2 \quad (1)$$

$$Total\ sugar\ concentration\ actual = -11.87117 + 5.18557A + 0.820001B + 0.470491C - 0.148370AB + 0.074333AC - 0.044822BC - 0.533203A^2 + 0.000465B^2 + 0.035412C^2 \quad (2)$$

Next, analysis of variance, commonly named ANOVA, is the analysis of a statistical model's collection that analyse the mean group and its variance [11]. Table 2 provides details on the analysis of variance (ANOVA) in the optimization of alkaline pre-treatment in producing sugar. The p-value for the quadratic model constructed was 0.0003. The Model F-value of 42.65 implied that the model was significant, with only a 0.03% chance that an F-value this large could occur due to noise occurrence. P-values of less than 0.050 indicate model terms are significant. In this case, the coefficients of A, C, AB, and BC were significant in the model terms. However, the insignificance of these three coefficients can be summarised as the lack of fit in the residual coefficient. The lack of fit F-value of 13.13 implied the lack of fit was significant. Thus, there was only a 2.23% chance that a lack of fit F-value this large could occur due to noise occurrence.

Table 2. Analysis of variance (ANOVA) for quadratic model

Source	**Sum of squares**	**Degree of freedom**	**Mean square**	**F-value**	**p-value**	**Remarks**
Model	101.11	9	11.23	42.65	0.0003	significant
A-NaOH concentration	22.31	1	22.31	84.7	0.0003	
B-Pretreatment time	0.0841	1	0.0841	0.3191	0.5966	
C-solid loading	3	1	3	11.39	0.0198	
AB	33.43	1	33.43	126.92	< 0.0001	
AC	0.4144	1	0.4144	1.57	0.2652	
BC	33.9	1	33.9	128.7	< 0.0001	
Residual	1.32	5	0.2634			
Lack of Fit	1.01	1	1.01	13.13	0.0223	significant
Pure Error	0.3075	4	0.0769			
Cor Total	102.43	14				

3.3 Interaction between Parameters

A three-dimensional (3D) response surface was used to observe and compare the interaction between parameters to reduce sugar production. Fig. 1(a) shows the 3D plot surface interaction between alkaline concentration (NaOH) and the pre-treatment time. The red region shows the maximum reducing sugar obtained with respect to the independent variables, whereas the blue region indicates the lowest reducing sugar production [12]. From the 3D plot interaction, a higher pre-treatment time results in better production of reduced sugar. However, the interaction between pre-treatment time and NaOH concentration shows that the higher NaOH concentration results in lower production of reduced sugar. The lower NaOH concentration does not influence the rice husk to change its structure, where the delignification and dissolution of hemicellulose occur at a lower reaction rate.

Next, Fig. 1(b) shows the 3D plot interaction between NaOH concentration and solid loading to reduce sugar production. Based on the graph, it can be analysed that lower solid loading results in higher reducing sugar production with lower NaOH concentration. Alkaline pre-treatment can occur in all rice husk structures when the solid loading is lower. Higher solid loading required a longer time to ensure that the entire structure of the rice husk was treated. In contrast, higher NaOH concentrations used for a large number of solid loadings declined since degradation of lignin cannot occur under high alkaline

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concentrations. Lignin degradation is more significant at lower NaOH concentrations or optimum conditions [13].

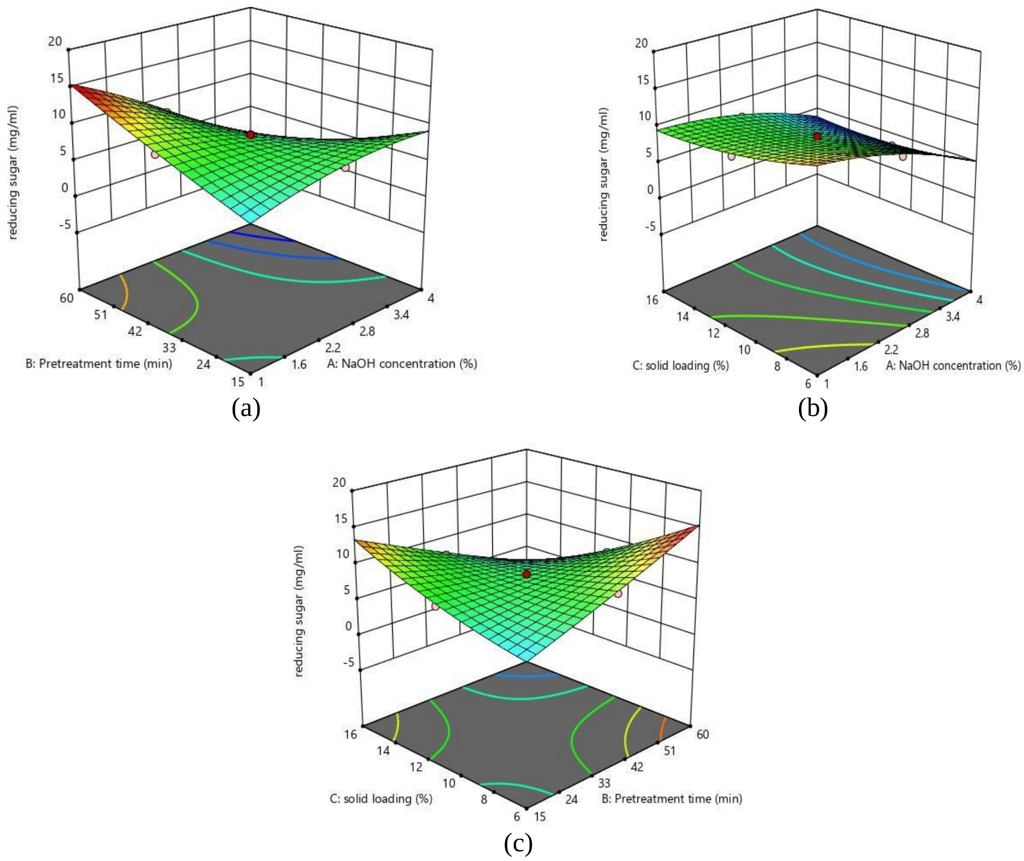

Fig. 1. 3D plot interaction between parameters: (a) NaOH concentration and pre-treatment time (b) NaOH concentration and solid loading (c) pre-treatment time and solid loading

Fig. 1(c) shows the 3D plot interaction between the pre-treatment time and the solid loading ratio of rice husk to produce reducing sugar. Higher solid loading can produce higher reducing sugar when a longer pre-treatment time is introduced to the pre-treatment. Higher pre-treatment time can enhance the rice husk's ability to undergo degradation of lignin and dissolution of hemicellulose. More cellulose content will be produced, which will utilise the production of reduced sugar. Research on the modelling of alkali pre-treatment of rice husk using RSM by researchers from Noshirvani University of Technology, Babol, Iran, was conducted in 2015. The effect of alkaline concentration, pre-treatment time and solid loading were analysed using the 3D modelling of RSM. From the study, it was analysed that increasing solid loading can increase the sugar yield. However, at a certain point, the sugar yield will be decreased at further higher solid loading. Thus, the optimal condition between these two variables is lower solid loading and higher pre-treatment time, where maximum reducing sugar was obtained.

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3.4 Actual and predicted graphical analysis

The predicted values of reducing sugar of each experimental run were calculated using Eq. (2), which indicates the coded value of predicted reducing sugar. Fig. 2 shows the bar chart on reducing sugar concentration of the experimental runs and predicted values from the RSM. Based on the bar chart shown, the highest value of reducing sugar concentration was obtained from run no 5. The sodium hydroxide concentration, pre-treatment time and solid loading ratio for this run are 4% w/v, 15 minutes and 16% w/v, respectively. The percentage error between the experimental runs and predicted values of reducing sugar concentration was low, where the average of this percentage error was 3.88%. This shows that the small value (less than 5%) of this percentage error indicates that the experimental runs were conducted successfully following the set parameter [14].

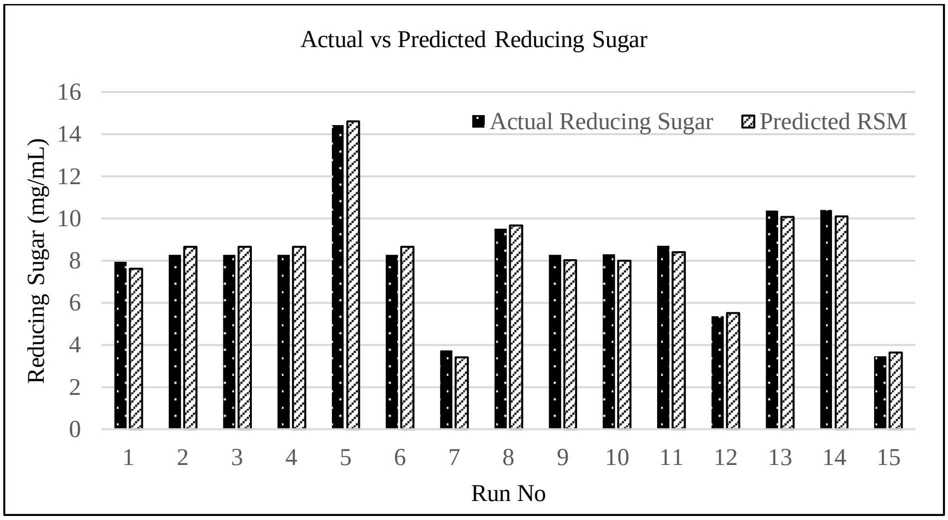

Fig. 2. Bar chart of the experimental runs and predicted values from the RSM Optimum parameter in producing higher reducing sugar

Table 3 shows the percentage error of the optimum value that achieves the highest reducing sugar concentration where the comparison can be analysed. The percentage error was calculated to determine whether the experimental design of optimization in alkaline pre-treatment is valid to the predicted model from RSM.

Table 3. Validation of the optimum value from RSM

Experimental runs (mg/mL)	Predicted value (mg/mL)	% Error
14.44	15.18	4.87

The validation of this experimental run can be expressed by the percentage error calculated using Eq. (3). The percentage error calculated is 4.87%, whereas the small margin (less than 5%) of error may be affected by the condition of the parameters set in the experiment. The optimum values of sodium hydroxide concentration, pre-treatment time and solid loading ratio are 1.67% w/v, 59.44 minutes and 7.67% w/v, respectively. The interaction concludes that the highest reducing sugar concentration can be obtained with lower alkaline concentration, longer pre-treatment time and average solid loading. Thus, this experimental optimisation run is valid following the independent variables set to the RSM.

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$$\% \text{ Error} = \frac{\text{Predicted value} - \text{Experimental value}}{\text{Predicted value}} \times 100\% \quad (3)$$

4. CONCLUSION

To improve the enzymatic saccharification of lignocellulosic biomass, sodium hydroxide, NaOH were used as an alkaline reagent. Alkaline pre-treatment is regarded as one of the most effective pre-treatment procedures, offering benefits in removing lignin from agricultural biomass. These include the rate of cellulose and hemicellulose solubilisation, the removal of lignin, the use of gentle reaction conditions, and the use of a low-cost procedure. RSM was used to optimise the parameter of alkaline pre-treatment, which is an important stage of this experiment. The optimisation stage for alkaline pre-treatment seeks to reduce the number of pre-treatment trials, thus reducing the number of tests to determine the ideal conditions of alkaline pre-treatment for the manufacture of reducing sugar. The optimum RSM values were obtained at 4%w/v NaOH concentration, 15 minutes of pre-treatment time, and 16%w/v solid loading, which produced the highest reducing sugar, 14.6 mg/mL. This value can be used to optimise alkaline pre-treatment in order to produce more xylose. However, at higher NaOH and longer time, it was reported that the pre-treatment caused the lignocellulosic materials to degrade to other unknown by-products, which resulted in lower production of reducing sugar [9]. Thus, the high amount of reducing sugar and xylose are essential components in the synthesis of various pharmaceuticals and bioproducts. Understanding their role in synthesis and treatment pathways can lead to improved yields, cost-effectiveness, and sustainability in the pharmaceutical and biotech industries.

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6. CONFLICT OF INTEREST STATEMENT

The authors declare that this research was conducted without any self-benefits, commercial or financial conflicts and that they have no conflicting interests with the funders.

7. AUTHORS' CONTRIBUTIONS

Siti Sabrina Mohd Sukri: Conceptualisation, supervision, writing – review and editing, and validation; **Nurul Khairani Aziz**: Conceptualisation, methodology and writing – original draft; **Wan Shahril Faizal Wan Yaacob**: Conceptualisation, methodology, formal analysis, investigation and writing – original draft.

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