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R Analysis for Optimizing Enzymatic Saccharification of Watermelon (*Citrullus lanatus*) Rind †

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Abstract: Watermelon waste was chosen as the main material due to the abundance of discarded rinds and the widespread consumption of this fruit in Malaysia. The cellulose, hemicellulose, and pectin within watermelon rinds, classified as lignocellulosic materials, underwent hydrolysis. This involved using cellulase and auxiliary enzymes like hemicellulose and pectinase to convert polysaccharides into simple sugars, yielding valuable end products. Thus, R software optimized saccharification yield in watermelon rind enzymatic hydrolysis. Four parameters were studied: substrate loading (1–5 g), enzyme loading (5–85 U/mg), temperature (35–55 °C), and hydrolysis time (6–30 h). Preliminary screening showed each parameter significantly affected saccharification yield. A mathematical model predicting optimal enzymatic hydrolysis conditions was developed through Response Surface Methodology (RSM) using Box–Behnken Design (BBD). The presented mathematical model exhibited a strong correlation between actual and predicted values, with a predicted R^2 value of 0.96%. Optimization led to conditions of 1.15 g substrate, 24.85 U/mg enzyme, 44.79 °C temperature, and 11.47 h hydrolysis time. Under these conditions, the actual saccharification yield of watermelon rind reached 70.72%.

Keywords: saccharification; enzymatic analysis; watermelon rinds; optimization; response surface methodology

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1. Introduction

Watermelon (*Citrullus lanatus*), a popular fruit known for its refreshing taste and high water content, generates a significant number of discarded rinds. Improper disposal and decomposition of these rinds can contribute to environmental issues [1]. The enzymatic breakdown of watermelon rind polysaccharides like cellulose, hemicellulose, and pectin produces fermentable sugars, which can be used to create biofuels and biobased compounds [2].

Efficient conversion of lignocellulosic biomass to fermentable sugars employs enzymatic saccharification, using specific enzymes to break down complex polysaccharides into simpler sugars [3]. However, research into enzymatic hydrolysis for sugar extraction from watermelon rinds is still lacking. Most studies have focused on chemical hydrolysis using acids or alkalis. Optimizing enzymatic saccharification methods is crucial to increase sugar yield and reduce bioconversion costs.

Moreover, no reports exist on using Response Surface Methodology (RSM) with R analysis to optimize enzymatic hydrolysis for lignocellulosic biomass waste. R, a powerful open-source programming language, is well-suited for statistical analysis, modeling

complex data, and creating predictive models [4,5]. This study concentrates on extracting sugars from watermelon rind through enzymatic hydrolysis. This approach has proven successful with other fruit peels. As watermelon rind constitutes a significant portion of the fruit's weight, converting it into value-added products presents a challenge.

Cellulase, hemicellulase, and pectinase enzymes from *Aspergillus niger* are used for enzymatic hydrolysis, breaking down polymeric carbohydrates into monomeric sugars [6]. The study optimizes enzymatic hydrolysis conditions such as substrate and enzyme loading, temperature, and hydrolysis time using R Studio version 3.2.5 and Response Surface Method (RSM) [7]. By enhancing watermelon rind utilization as a valuable bioresource, the study transforms agricultural waste into useful products and promotes sustainable biorefinery practices.

This research is crucial because it addresses a significant environmental challenge by transforming watermelon rind, a commonly discarded agricultural byproduct, into fermentable sugars that can be used to produce biofuels and other value-added products. By focusing on enzymatic saccharification, it promotes an eco-friendly alternative to chemical hydrolysis, reducing the reliance on hazardous chemicals and minimizing environmental harm. Furthermore, the novel application of RSM in R for process optimization not only improves efficiency but also contributes to sustainable biorefinery practices, aligning with global efforts toward a circular economy and renewable energy production. Ultimately, this study offers innovative solutions for waste management and adds value to agricultural residues, supporting the development of sustainable and cost-effective bioresources.

2. Materials and Methods

2.1. Materials

The unused and unwanted watermelon rinds, which served as raw material in this research, were collected from the university's café (Universiti Sains Malaysia, Pulau Pinang, Malaysia). The enzymes used were cellulase from Aspergillus sp (0.3 units/mg), pectinase from Aspergillus niger (>1 U/mg), and hemicellulase from Aspergillus niger (0.3–3.0 units/mg solid), employing the β-Galactose dehydrogenase system and locust gum as the substrate. The chemicals utilized included anthrone reagent, chloramphenicol (98%), tetracycline hydrochloride (secondary standard), acetic acid, and sodium acetate. All enzymes and chemicals originated from the same source: Sigma-Aldrich, St. Louis, MO, USA, Merck KGaA, Darmstadt, Germany.

2.2. Methods

2.2.1. Preparation of Watermelon Rinds (Substrate)

The watermelon rinds were cleaned with tap water and cut into small pieces, approximately 1 cm in length and 0.5 cm thick. The rinds were then dried in an oven at 70 °C for 24 h until completely dry [8]. The dried watermelon peel pieces were ground using a kitchen blender. The substrate was subsequently sieved using an Endecotts sieve (BS 410-1-Endecotts, London, UK), ensuring particle size remained below 90 µm [9].

2.2.2. Determination of the Most Feasible Enzymes for Enzymatic Hydrolysis of Watermelon Rinds

Each substrate, weighing 1.0 g, was placed into a 250 mLshake flask and combined with 100 mLof 0.05 M sodium acetate buffer solution at pH 4.8. The enzyme loadings used were 25 U/mg for individual enzymes (cellulase, hemicellulase, pectinase), a combination of both (pectinase + cellulase), and a combination of three enzymes (pectinase, hemicellulase, and cellulase). Additionally, 30 µg/ mLeach of chloramphenicol and tetracycline hydrochloride were added to prevent microbial contamination [8]. The flasks were placed in an incubator

shaker (IKA® KS 4000i, IKA Werke GmbH & Co. KG, Staufen im Breisgau, Germany) at 45 °C and 100 rpm for 24 h. Each taken sample was placed in an oven for 15 min at 105 °C for enzyme inactivation. The samples were kept overnight in the chiller at 6 °C before undergoing the analysis of reducing sugar.

2.2.3. Optimization of Hydrolysis Condition of Watermelon Rinds by Using RSM

The effect and interaction between factors on saccharification yield (%) to optimize the hydrolysis conditions of watermelon rinds were investigated using the RSM package in R. The four factors involved were substrate loading (1–5 g), enzyme loading (5–85 U/mg), temperature (35–55 °C), and hydrolysis time (6–30 h). The screening test was performed using one-way analysis of variance (ANOVA) in the R software version 3.2.5 to determine the significance of variables at a confidence level greater than 95%. Significant factors with p-values less than 0.05 were then further investigated using RSM in a Box–Behnken design (BBD). Following ANOVA and regression fitting, the significant independent variables and responses were fitted into a second-order polynomial equation that was used to anticipate the optimal conditions for saccharification yield (%).

2.2.4. Determination of Total Reduced Sugar

The quantity of reduced sugars released through enzymatic hydrolysis was determined using the dinitrosalicylic acid (DNS) method [10]. To perform the reducing sugar analysis, the sample was centrifuged at 4 °C and 3500 rpm for 10 min using a centrifuge machine (centrifuge model 5500, KUBOTA Corporation, Osaka, Japan). Subsequently, 1.0 mLof supernatant was mixed with 2.0 mLof DNS reagent and boiled for 5 min on a hot plate (IKA Werke GmbH & Co. KG, Staufen im Breisgau, Germany). After boiling, the sample was allowed to cool to room temperature. The concentration of reduced sugar was measured at 575 nm using a spectrophotometer (Hitachi U-1900, American Laboratory Trading, East Lyme, CT, USA), with glucose serving as the standard. The quantity of reduced sugar was determined using a glucose standard curve and expressed as a percentage of saccharification of sugar using Equation (1) [9].

$$Saccharification\ yield\ (\%) = \frac{\left(Glucose_f - Glucose_i\right)\left(\frac{mg}{ml}\right) \times 100}{Substrate\ \left(\frac{mg}{ml}\right)} \quad (1)$$

where the $Glucose_f$ represents glucose concentration at the final and $Glucose_i$ represents the glucose concentration at the initial in mg/mL.

3. Results and Discussion

3.1. Effect of Different Types of Enzyme Treatment

Enzymes were tested individually and in combinations of two or three. Cellulase was most effective when used alone, yielding 23.04% saccharification of watermelon rinds' polysaccharides, outperforming hemicellulase and pectinase. Combining enzymes led to higher yields than using one type. The results showed almost a 30% difference between the low (12.28%) and high (41.02%) yields, likely due to enzyme type/amount and watermelon rind components (hemicellulose, cellulose, pectin). Gama (2013) [11] suggested that enzyme combinations increase hydrolysis rate and synergy. Prior research [8] found that combining pectinase and cellulase degraded polysaccharides better than pectinase alone. Andlar et al. (2018) [12] explained that 'synergy' enhances the breaking down of lignocellulose when enzymes cooperate.

3.2. Screening of Parameters Affecting Saccharification Yield for Enzymatic Hydrolysis of Watermelon Rinds

The study assessed four factors (substrate loading, enzyme loading, temperature, and hydrolysis time) to determine optimal saccharification yields. Initial screening set the parameters at 1g substrate, 25 U/mg enzyme, 45 °C, and 12 h hydrolysis. A lower substrate (5 g) resulted in an 8.7% yield, while 1 g led to a 40.33% yield. A higher substrate hindered conversion due to factors like end-product inhibition and mass transfer limitations [13]. Optimal enzyme loading was 25 U/mg, yielding 40.04%, as excessive enzymes led to yield reduction due to saturation and inhibition [14,15]. A higher temperature (45 °C) increased yield (67.15%) due to improved enzyme–substrate bonding, but a lower temperature (35 °C) hindered metabolic function and yield [16]. Temperatures beyond 45 °C decreased yield due to toxic byproducts [16]. Saccharification yield increased from 6 to 12 h, peaking at 67.39%, then declined due to altered substrate structure, enzyme cooperation, and eventual inhibition [17,18]. Longer hydrolysis-generated inhibitors, like furfural and hydroxymethylfurfural (HMF), can reduce yield [19]. All parameters significantly affected yield (p-value < 0.05) per ANOVA [Table 1], justifying further optimization using R software version 3.2.5.

Table 1. Analysis of factors that affect enzyme hydrolysis using one-way ANOVA.

	Df	Sum Sq	Mean Sq	F-Value	Prob (>F)
Substrate loading	4	1975.6	489.4	945.7	7.63×10^{-13} ***
Enzyme loading	4	1764.8	441.2	516.9	1.54×10^{-11} ***
Temperature	4	3141.4	785.3	274.7	3.55×10^{-10} ***
Hydrolysis time	4	3682	920.5	331.1	1.41×10^{-10} ***

Significant codes: 0 '***'.

3.3. Optimization of Immobilization Parameter

The highest saccharification yield was observed at a substrate loading of 1 g (40.33%), enzyme loading of 25 U/mg (40.04%), temperature of 45 °C (67.15%), and hydrolysis time of 12 h (67.36%). These values were then used as central values for BBD, resulting in thirty experiments yielding the highest saccharification yield (69.89%) and the lowest saccharification yield (40.36%). To establish the statistical models, the experimental results from BBD underwent ANOVA and regression fitting. The significance of each coefficient was determined by the probability F (p-value) values at a 95% confidence level, deciding whether to reject the null hypothesis. Smaller p-values indicate greater statistical significance, aligning with Breen's explanation [20]. Table 2 displays enzyme activity in response to input variable combinations as per the experimental design. A lack of fit F-value of 1.6594 suggests that the "lack of fit" is not significantly different from pure error. In other words, there is a 30% chance that this lack of fit F-value is due to random noise [21]. As stated by Sariman [22], a high R^2 value indicates a strong correlation among independent variables, making it an excellent basis for estimating optimal conditions to maximize the dependent variable. The R^2 value of 0.96 for the second-order model indicates that the model explains over 96% of the variance in the response, with less than 4% unaccounted. The model's validity is further confirmed by the close alignment between R^2 values (0.96) and adjusted R^2 values (0.9226). Significant variables from the multiple regression analysis of BBD experiments were fitted to a second-order polynomial model (Equation (2)), illustrating the relationships between independent variables and their effects on the responding variables.

$$Y = 68.2766 - 1.5425X_1 + 1.3650X_2 - 2.0366X_4 - 9.5229X_1^2 - 7.2341X_2^2 - 3.2804X_3^2 - 4.3141X_4^2 - 6.1125X_1\ X_2 - 4.5750X_1\ X_3 - 5.5550X_1\ X_4 \quad (2)$$

where, X_1 is the substrate loading (g), X_2 is the enzyme loading (U/mg), X_3 is the temperature (°C), and X_4 is the hydrolysis time.

Table 2. ANOVA and regression fitting of process parameters for enzymatic hydrolysis.

	Polynomial Coefficient	**Std. Error**	**t-Value**	***p*-Value Prob(>|t|)**
(Intercept)	68.2766	0.8047	84.8478	$<2.2 \times 10^{-16}$ ***
X_1	1.5425	0.5690	2.7109	0.0161 *
X_2	1.3650	0.5690	2.3989	0.0298 *
X_3	−0.5925	0.5690	−1.0413	0.3142
X_4	−2.0366	0.5690	−3.5793	0.0027 **
X_1X_2	−6.1125	0.9855	−6.2021	1.693×10^{-5} ***
X_1X_3	−4.5750	0.9855	−4.6421	0.0003192 ***
X_1X_4	−5.5550	0.9855	−5.6365	4.734×10^{-5} ***
X_2X_3	1.8925	0.9855	1.9203	0.0740
X_2X_4	−1.8050	0.9855	−1.8315	0.0869
X_3X_4	1.0550	0.9855	1.0705	0.3013
X_1^2	−9.5229	0.7527	−12.6513	2.092×10^{-9} ***
X_2^2	−7.2341	0.7527	−9.6106	8.404×10^{-8} ***
X_3^2	−3.2804	0.7527	−4.3581	0.0005622 ***
X_4^2	−4.3141	0.7527	−5.7314	3.971×10^{-5} ***
Lack of fit			1.6594	0.3000
Predicted R^2	0.96			
Adjusted R^2	0.9226			
F-statistic	25.7			

Significant codes: '***' 0.001; '**' 0.01; '*' 0.05.

3.4. The Effect of Substrate and Enzyme Loading on Saccharification Yield

Both substrate loading (2.7109) and enzyme loading (2.3989) exhibit positive F-values (Table 2), with substrate loading slightly higher. This suggests that substrate loading has a greater impact on saccharification yield compared with enzyme loading. The reciprocal influence of factors on saccharification yield is best depicted in Figure 1, utilizing three-dimensional surface and contour plots. In accordance with the response surface plot in Figure 1a, substrate loading and enzyme loading mutually influence each other in watermelon rind hydrolysis. At the lowest substrate loading (0.5 g) and enzyme loading (20 U/mg), the saccharification yield is approximately 40%. As substrate loading is increased to 1 g and enzyme loading to 25 U/mg, the saccharification yield surpasses 60%. This outcome indicates a strong influence of substrate loading and enzyme loading on saccharification yield. The figure illustrates an upward trend in saccharification yield as substrate loading increases from 0.5 g to 1.0 g. However, further increases beyond 1.2 g result in a decline in saccharification yield. This trend differs from Gama's findings [11] on apple pomace hydrolysis, in which increased substrate concentration led to heightened sugar release. The trend in enzyme loading aligns with Rivera's observations [23], who noted that elevated enzyme loadings did not necessarily yield higher sugar release from sugarcane bagasse. As substrate loading rises, the viscosity of the watermelon slurry also increases, hindering mass transfer and contributing to reduced sugar conversion due to lower enzyme loading [24]. Figure 1a presents a contour plot illustrating the combined effects of substrate loading and enzyme loading on saccharification yield. The plot highlights that maintaining substrate loading and enzyme loading at the center (depicted in reddish tones) yields a high saccharification yield.

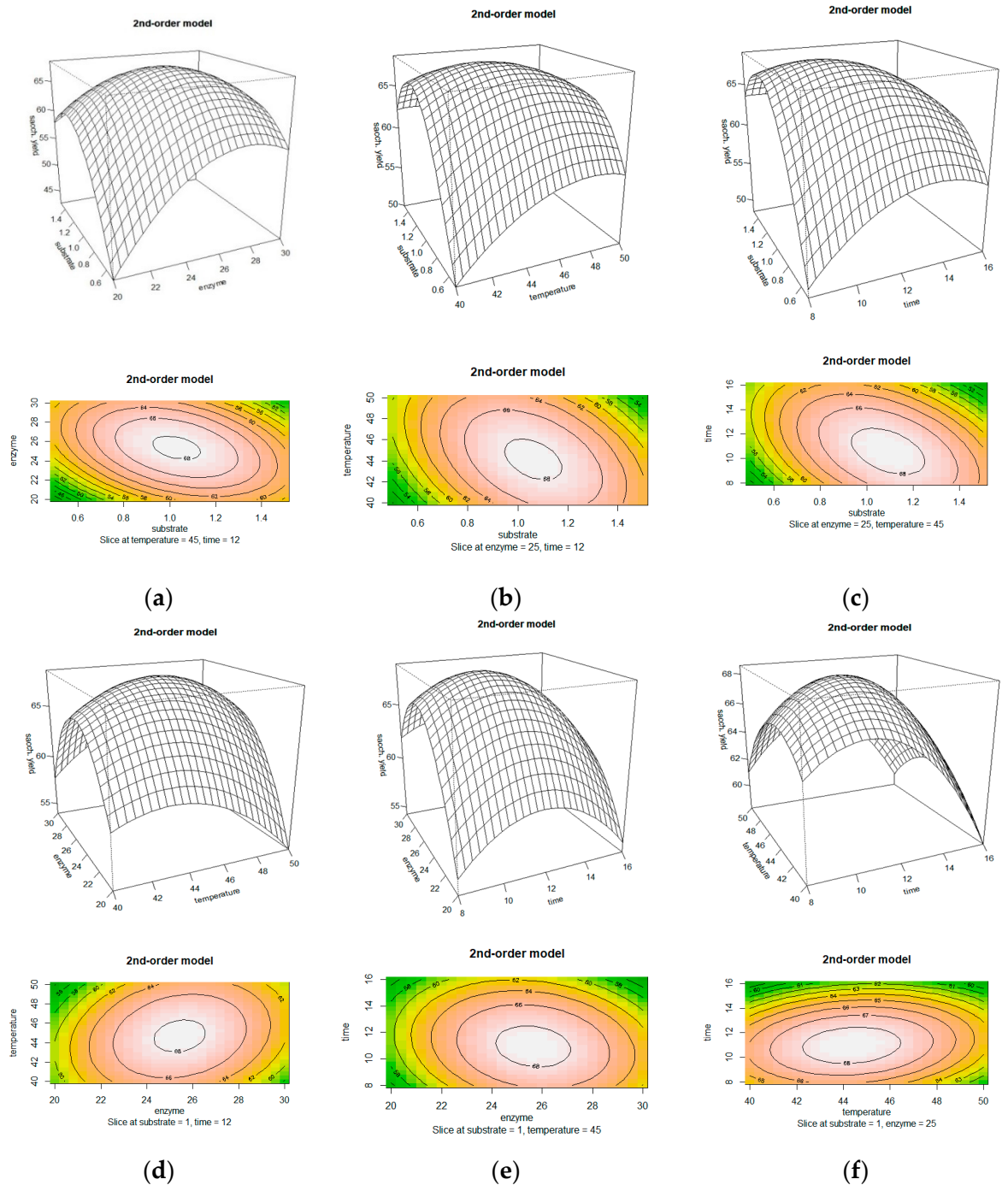

Figure 1. Response surface plot and contour plot for the interaction between independent variables (**a**) substrate and enzyme loading, (**b**) substrate loading and temperature, (**c**) substrate loading and hydrolysis time, (**d**) enzyme loading and temperature, (**e**) enzyme loading and hydrolysis time, and (**f**) temperature and hydrolysis time.

3.5. The Effect of Substrate Loading and Incubation Temperature on Saccharification Yield

ANOVA analysis indicated that substrate loading had a greater impact on saccharification yield compared to temperature, as evidenced by its higher F-value (2.7109) in contrast to temperature's (−1.0413). Furthermore, the interaction between substrate loading and temperature yielded a negative F-value (−4.6421) (Table 2), signifying an antagonistic relationship between these factors. The response surface plot in Figure 1b underscores that reducing the temperature and increasing the substrate loading results in a higher yield. However, once the local limit of fructose conversion is reached, typically occurring

at a substrate loading of 1 g and a temperature of 40–45 °C, saccharification yield starts decreasing progressively. The optimal saccharification yield (92.43%) is observed in the reddish area of Figure 1b, with a substrate loading of 1–1.2 g and a temperature of 45–50 °C. To be more precise, the maximum yield (92.43%) of fructose oleate was achieved at a molar ratio of 1:3.3 and a temperature of 55 °C. At temperatures of 45 °C and below, the plot appears in yellow and green tones, indicating lower sugar conversion. This is because, as mentioned by Kennes [25], lower temperatures can often impede or inhibit the activity of enzymes or other catalysts involved in the conversion of sugars or other compounds.

3.6. The Effect of Substrate Loading and Hydrolysis Time on Saccharification Yield

Both substrate loading (p-value = 2.2×10^{-16}) and hydrolysis time (p-value = 0.0027) significantly impact the response variables, as evidenced by their p-values being less than 0.05 (Table 2). Substrate loading has a more pronounced effect on saccharification yield than hydrolysis time, with its F-value (2.7109) exceeding that of hydrolysis time (−1.0413). Referring to the response surface plot in Figure 1c, the impact of substrate loading and hydrolysis time on saccharification yield is depicted, while enzyme loading (25 U/mg) and temperature (45 °C) remain fixed. The figure illustrates that saccharification yield increases with the rise in substrate loading (0.5 g to 1.0 g), but further increments to 1.5 g result in a decline. Increasing substrate loading augments watermelon slurry viscosity, limiting mass transfer and subsequently the reduced sugar conversion due to the relatively low enzyme loading [24]. The saccharification yield also rises with longer hydrolysis time (8 to 12 h) but extending to 16 h causes a slight reduction in yield. The decline in hydrolysis rate over time is attributed to a notable loss of enzyme activity during the incubation period, even if the initial substrate loading ensured enzyme saturation [18]. Figure 1c presents a contour plot depicting the combined effects of substrate loading and hydrolysis time on saccharification yield. It is evident from the plot that maintaining substrate loading and hydrolysis time at the center (depicted in reddish tones) yields a high saccharification yield. The optimal saccharification yield (92.43%) is observed in the reddish area of Figure 1c, with a substrate loading of 0.8–1.0 g and hydrolysis time of 10–12 h.

3.7. The Effect of Enzyme Loading and Incubation Temperature on Saccharification Yield

Table 2 indicates that enzyme loading has a significant effect on saccharification yield, with a p-value of 0.0161, while temperature does not exhibit a significant effect (p-value = 0.3142). The corresponding F-values affirm this distinction, with enzyme loading (2.3989) exerting a more substantial influence on the reaction compared to temperature (−1.0413). The response surface plot in Figure 1d depicts how reducing enzyme loading (20 U/mg) and elevating temperature (50 °C) lead to a decrease in saccharification yield. Similarly, the contour plot in Figure 1d highlights the lowest yield in the green area, characterized by the highest temperature and lowest enzyme loading. The highest saccharification yield is evident within the reddish area, corresponding to enzyme loading in the range of 24–25 U/mg and temperatures between 44 °C and 46 °C. These findings align with those of Pandiyan [26], where increased temperature resulted in diminished saccharification yield. Notably, an enhancement in saccharification yield was observed with elevated enzyme loading.

3.8. The Effect of Enzyme Loading and Hydrolysis Time on Saccharification Yield

Both enzyme loading (p-value = 0.0298) and hydrolysis time (p-value = 0.0027) exert significant effects on saccharification yield (Table 2). However, enzyme loading holds a higher F-value (2.3989), signifying that its impact on saccharification yield is greater than that of hydrolysis time (−3.5793). Based on the response surface plot in Figure 1e, an increase in enzyme loading leads to a higher saccharification yield when hydrolysis time

is maintained at the center (12 h). However, saccharification yield decreases as enzyme loading surpasses 25 U/mg. Figure 1e also displays a contour plot illustrating the effects of enzyme loading and hydrolysis time on saccharification yield. The plot indicates that a high saccharification yield can be achieved by maintaining enzyme loading within the range of 24–26 U/mg and a hydrolysis time of 8–12 h within the reddish area. Zhu [27] suggested that extending the reaction time can promote a more complete hydrolysis of polysaccharides, leading to a higher saccharification yield. Nonetheless, there might be diminishing returns at some point, as the reaction could reach equilibrium or face other limiting factors.

3.9. The Effect of Incubation Temperature and Hydrolysis Time on Saccharification Yield

Both temperature (−1.0413) and hydrolysis time (−3.5793) exhibit negative F-values (Table 2). However, when considering *p*-values, hydrolysis time (*p*-value = 0.0027) demonstrates a more significant effect on saccharification yield compared to temperature (*p*-value = 0.3142). In accordance with the response surface plot depicted in Figure 1f, decreasing both temperature and hydrolysis time leads to a reduction in saccharification yield. The highest saccharification yield is observed at the center values of temperature (44–46 °C) and time (10–12 h). Furthermore, the contour plot illustrates that the reddish zone corresponds to greater sugar conversion, with temperature and time maintained at their central levels [28]. However, both Figure 1f diagrams indicate that a higher temperature and longer hydrolysis time result in slower reaction kinetics, leading to a lower saccharification yield.

3.10. Attaining Optimum Conditions and Model Validation

The optimal conditions for maximizing enzyme activity were determined using the desirability function, a feature provided by one of the R software packages. The resulting desirability value was calculated as 0.9454, and the optimal conditions identified through the desirability mechanism were as follows: substrate loading of 1.15 g, enzyme loading of 24.85 U/mg, temperature of 44.79 °C, and a hydrolysis time of 11.47 h, which resulted in an enzyme activity of 69.28%. To validate the model and the predicted optimum value, triplicate validation experiments were conducted at the projected conditions. Surpassing expectations, the experimental outcomes yielded a 70.72% saccharification yield, demonstrating the model's reliability in forecasting the optimal yield for enzymatic hydrolysis of watermelon rinds. The precision of the model was further affirmed by the close alignment of the experimental values with the predicted outcomes.

4. Conclusions

Watermelon rinds stand out as a cost-effective and promising feedstock for generating value-added products. Achieving a high saccharification yield involved synergistically combining various enzymes, including cellulase, hemicellulase, and pectinase, within the watermelon rind slurry, ensuring efficient enzymatic hydrolysis. The initial screening phase revealed the significant impact of substrate loading, enzyme loading, temperature, and hydrolysis time on yield. Subsequently, the Box–Behnken design of Response Surface Methodology (RSM), executed using R software, optimized these parameters. The second-order polynomial model exhibited a predicted R^2 value of 0.96 and a significant probability value ($p < 0.001$), confirming the model's reliability. The optimal conditions—1.15 g substrate, 24.85 U/mg enzyme, 44.79 °C, and 11.47 h—resulted in a saccharification yield of 70.72%, closely aligning with predicted values. This eco-friendly approach offers a sustainable solution for waste valorization and bioresource development.

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References

1. Amaeze, N.; Okoliegbe, I.; Francis, M. Cellulase production by Aspergillus niger and Saccharomyces cerevisiae using fruit wastes as substrates. *Int. J. Appl. Microbiol. Biotechnol.* **2015**, *3*, 36–44.
2. Kamusoko, R.; Jingura, R.M.; Parawira, W.; Chikwambi, Z. Strategies for valorization of crop residues into biofuels and other value-added products. *Biofuels Bioprod. Biorefining* **2021**, *15*, 1950–1964. [CrossRef]
3. Periyasamy, S.; Isabel, J.B.; Kavitha, S.; Karthik, V.; Mohamed, B.A.; Gizaw, D.G.; Aminabhavi, T.M. Recent advances in consolidated bioprocessing for conversion of lignocellulosic biomass into bioethanol—A review. *Chem. Eng. J.* **2023**, *453*, 139783. [CrossRef]
4. Sampaio, P.S.; Fernandes, P. Machine Learning: A Suitable Method for Biocatalysis. *Catalysts* **2023**, *13*, 961. [CrossRef]
5. Kim, H. Propensity score analysis in non-randomized experimental designs: An overview and a tutorial using R software. *New Dir. Child Adolesc. Dev.* **2019**, *2019*, 65–89. [CrossRef] [PubMed]
6. Poshina, D.N.; Raik, S.V.; Poshin, A.N.; Skorik, Y.A. Accessibility of chitin and chitosan in enzymatic hydrolysis: A review. *Polym. Degrad. Stab.* **2018**, *156*, 269–278. [CrossRef]
7. Kassim, M.A.; Hussin, A.H.; Meng, T.K.; Kamaludin, R.; Zaki MS, I.M.; Zakaria WZ, E.W. Valorisation of watermelon (*Citrullus lanatus*) rind waste into bioethanol: An optimization and kinetic studies. *Int. J. Environ. Sci. Technol.* **2022**, *14*, 8671–8680. [CrossRef]
8. Hasem, N.H.; Mohamad Fuzi SF, Z.; Kormin, F.; Abu Bakar, M.F.; Sabran, S.F. Extraction and partial characterization of durian rind pectin. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *269*, 012019. [CrossRef]
9. Suhag, M.; Kumar, A.; Singh, J. Saccharification and fermentation of pretreated banana leaf waste for ethanol production. *SN Appl. Sci.* **2020**, *2*, 1448. [CrossRef]
10. Sahariah, P.; Másson, M. Antimicrobial chitosan and chitosan derivatives: A review of the structure–activity relationship. *Biomacromolecules* **2017**, *18*, 3846–3868. [CrossRef]
11. Gama, R. A Lignocellulolytic Enzyme System for Fruit Waste Degradation: Commercial Enzyme Mixture Synergy and Bioreactor Design. Ph.D. Dissertation, Rhodes University, Grahamstown, South Africa, 2013.
12. Andlar, M.; Rezić, T.; Marđetko, N.; Kracher, D.; Ludwig, R.; Šantek, B. Lignocellulose degradation: An overview of fungi and fungal enzymes involved in lignocellulose degradation. *Eng. Life Sci.* **2018**, *18*, 768–778. [CrossRef] [PubMed]
13. Gaikwad, A. Effect of particle size on the kinetics of enzymatic hydrolysis of microcrystalline cotton cellulose: A modeling and simulation study. *Appl. Biochem. Biotechnol.* **2019**, *187*, 800–816. [CrossRef] [PubMed]
14. Payne, C.M.; Knott, B.C.; Mayes, H.B.; Hansson, H.; Himmel, M.E.; Sandgren, M.; Beckham, G.T. Fungal cellulases. *Chem. Rev.* **2015**, *115*, 1308–1448. [CrossRef]
15. Alrumman, S.A. Enzymatic saccharification and fermentation of cellulosic date palm wastes to glucose and lactic acid. *Braz. J. Microbiol.* **2016**, *47*, 110–119. [CrossRef]

16. Gao, Z.; Alshehri, K.; Li, Y.; Qian, H.; Sapsford, D.; Cleall, P.; Harbottle, M. Advances in biological techniques for sustainable lignocellulosic waste utilization in biogas production. *Renew. Sustain. Energy Rev.* **2022**, *170*, 112995. [CrossRef]
17. Copley, S.D. Shining a light on enzyme promiscuity. *Curr. Opin. Struct. Biol.* **2017**, *47*, 167–175. [CrossRef]
18. Pramanik, S.K.; Mahmud, S.; Paul, G.K.; Jabin, T.; Naher, K.; Uddin, M.S.; Saleh, M.A. Fermentation optimization of cellulase production from sugarcane bagasse by *Bacillus pseudomycoides* and molecular modeling study of cellulase. *Curr. Res. Microb. Sci.* **2021**, *2*, 100013. [CrossRef]
19. Zou, X.; Jiang, X.; Wen, Y.; Wu, S.; Nadege, K.; Ninette, I.; Wang, X. Enzymatic synthesis of structured lipids enriched with conjugated linoleic acid and butyric acid: Strategy consideration and parameter optimization. *Bioprocess Biosyst. Eng.* **2020**, *43*, 273–282. [CrossRef]
20. Breen, E.J.; Tan, W.; Khan, A. The statistical value of raw fluorescence signal in Luminex xMAP based multiplex immunoassays. *Sci. Rep.* **2016**, *6*, 26996. [CrossRef]
21. Behera, S.K.; Meena, H.; Chakraborty, S.; Meikap, B.C. Application of response surface methodology (RSM) for optimization of leaching parameters for ash reduction from low-grade coal. *Int. J. Min. Sci. Technol.* **2018**, *28*, 621–629. [CrossRef]
22. Sariman, S.N.S.A.; Zakaria, W.Z.E.W.; Serri, N.A. The optimization of fructose oleate ester in packed bed reactor (PBR) using R analysis. *AIP Conf. Proc.* **2023**, *2785*, 040007. [CrossRef]
23. Rivera, E.C.; Rabelo, S.C.; dos Reis Garcia, D.; da Costa, A.C. Enzymatic hydrolysis of sugarcane bagasse for bioethanol production: Determining optimal enzyme loading using neural networks. *J. Chem. Technol. Biotechnol.* **2010**, *85*, 983–992. [CrossRef]
24. Nadar, S.S.; Rao, P.; Rathod, V.K. Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review. *Food Res. Int.* **2018**, *108*, 309–330. [CrossRef] [PubMed]
25. Kennes, D.; Abubackar, H.N.; Diaz, M.; Veiga, M.C.; Kennes, C. Bioethanol production from biomass: Carbohydrate vs. syngas fermentation. *J. Chem. Technol. Biotechnol.* **2016**, *91*, 304–317. [CrossRef]
26. Pandiyan, K.; Tiwari, R.; Singh, S.; Nain, P.K.; Rana, S.; Arora, A.; Nain, L. Optimization of enzymatic saccharification of alkali pretreated *Parthenium* sp. using response surface methodology. *Enzym. Res.* **2014**, *2014*, 764898. [CrossRef]
27. Zhu, J.Y.; Pan, X. Efficient sugar production from plant biomass: Current status, challenges, and future directions. *Renew. Sustain. Energy Rev.* **2022**, *164*, 112583. [CrossRef]
28. Mettler, M.S.; Mushrif, S.H.; Paulsen, A.D.; Javadekar, A.D.; Vlachos, D.G.; Dauenhauer, P.J. Revealing pyrolysis chemistry for biofuels production: Conversion of cellulose to furans and small oxygenates. *Energy Environ. Sci.* **2012**, *5*, 5414–5424. [CrossRef]