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Improved Sugar Recovery from Orange Peel by Statistical Optimization of Thermo-Alkaline Pretreatment

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Abstract: Orange peel, which is a by-product of oranges, contains carbohydrates that can be converted into sugars and used in the fermentation process. In this study, the thermal alkaline pretreatment process was chosen because of its simplicity and lesser reaction time. In addition, the reaction factors were optimized using response surface methodology. The determined optimal conditions were as follows: 60.1 g/L orange peels loading, 3% KOH and 30 min. Under the optimal conditions, glucan content (GC) and enzymatic digestibility (ED) were found to be 32.8% and 87.8%, respectively. Enzymatic hydrolysis was performed with pretreated and non-pretreated orange peels using three types of enzyme complex (cellulase, cellobiase and xylanase). The minimum concentrations of enzyme complex required to obtain maximum ED were 30 FPU (filter paper unit), 15 CBU (cellobiase unit), and 30 XNU (xylanase unit) based on 1 g-biomass. Additionally, ED of the treated group was approximately 3.7-fold higher than that of the control group. In conclusion, the use of orange peel as a feedstock for biorefinery can be a strategic solution to reduce wastage of resources and produce sustainable bioproducts.

Keywords: alkaline; biomass; enzymatic hydrolysis; optimization; pretreatment

1. Introduction

Rising global population is leading to increasing food production and consumption, eventually leading to growing food wastage [1–3]. These food wastes affect public health as well as the environment because they are disposed of by landfilling or incineration [4,5]. Sustainable development has been remarked to deal with environmental concerns and economic growth in the long term [6–8]. To this end, a biorefinery system has received considerable attention, as it is a continuous eco-friendly process that uses renewable biomass, such as food waste and microalgae [9,10]. As food waste is a sugar-rich biomass, the biorefinery system focuses on the utilization of water-soluble carbohydrates that are abundant in food wastes [11–14]. A biorefinery uses carbohydrates from biomass to produce value-added materials through microbial fermentation, but these carbon sources act as a high-cost factor in the fermentation process [15]. Therefore, in order to scale-up the process, carbon sources need to be replaced with inexpensive biomass.

According to the Food and Agriculture Organization (FAO), annual food waste, which have the potential to be next-generation biomass, was estimated at about 1.3 billion tons [16]. In addition, 40–50% of food wastes consist of fruit and vegetable waste [17]. Worldwide, orange is one of the most cultivated fruits. In 2018, approximately 75 million tons of oranges were produced globally; moreover, 50–60% (*w/w*) of these oranges consisted of residues (peels, seeds, and membrane parts), which were generated after processing [18,19].

Orange peels consist of cellulose, hemicellulose, and pectin [20,21]. Various pretreatment processes, such as physical, chemical, biological pretreatment, have been applied to increase the enzyme accessibility and to increase the conversion of carbohydrate to sugar from biomass [9,22]. Chemical pretreatments (acid and alkaline) are commonly used to improve enzyme accessibility [23]. Although acid pretreatment is an appropriate method

to solubilize carbohydrates, it leads to the loss of solubilized carbohydrates, which reduces commercial feasibility [24,25]. In addition, acid can corrode equipment, leading to considerable cost [26]. Thus, it is not ideal for a scale-up process. Alkaline pretreatment increases the accessibility of enzyme to cellulose and hemicellulose [9]. However, they have a long reaction time. For industrial application, a combination of thermal pretreatment process is important to shorten the reaction time [27]. It has been reported that the simultaneous use of diverse pretreatments is effective to increase the degradability of biomass [23,28].

Pretreatment, which accounts for ~35% of the total production cost, is an important step in order to utilize the carbohydrates in biomass [29,30]. To design an economical process, it is required to use the statistical method such as response surface methodology (RSM) [31]. RSM provides an alternative methodology by considering the reciprocal connection between variables based on the experimental responses and provides an estimate of the integrated effect of these variables [32].

Enzymes are required to hydrolyze carbohydrates, such as cellulose and hemicellulose, into monosaccharides [33]. In particular, in an orange peel, cellulase hydrolyzes β-1,4-glycosidic bonds into cellulose and cellobiase hydrolyzes cellobiose or oligosaccharides from the non-reducing ends to glucose [34]. Xylanase is used for hydrolysis of xylan, which is a xylose biopolymer [35]. Optimized reaction conditions and enzyme loading were needed to investigate efficient enzyme hydrolysis [36].

In this study, orange peel underwent thermo-alkaline pretreatment to improve sugar production. The reaction conditions for KOH pretreatment of biomass were statistically optimized using the RSM. In addition, profiling of enzymatic hydrolysis was investigated to determine the efficiency of enzyme loading and reaction time. Finally, the overall process of sugar conversion from biomass was evaluated using a material balance based on 1000 g of orange peel.

2. Materials and Methods

2.1. Materials

Oranges were purchased from Sandlejeoung (Andong, Korea). Celluclast® 1.5L and C-tec 2 were purchased from Novozymes (Krogshoejvej, Bagsværd, Denmark). Xylanase (xylanase from *Trichoderma viride*) was purchased from Honeywell Fluka (Morris Plains, NJ, USA). Potassium hydroxide (KOH), sulfuric acid (H_2SO_4), and calcium carbonate ($CaCO_3$) were purchased from Samchun Chemical (Kangnam-Gu, Seoul, South Korea). Citric acid trihydrate was purchased from Junsei (Chuo-ku, Tokyo, Japan).

2.2. Raw Material and Alkaline Pretreatment

The orange peel was air-dried at 105 °C in an oven for 12 h. For the pretreatment, it was ground to 90–100 μm size and autoclaved at 121 °C using KOH. After the pretreatment, the pretreated orange peel was neutralized using de-ionized water (DW) and then dried in an oven at 105 °C for 12 h.

2.3. Design of Experiment by Response Surface Methodology (RSM)

Central composite design (CCD) of RSM was carried out to optimize the significant variables affecting alkaline pretreatment (X_1: time, X_2: KOH concentration, and X_3: solid loading). CCD demonstrates a mathematical model of the interaction of independent variables on alkaline pretreatment for enzymatic hydrolysis. Table 1 shows the variables and their different levels in order to optimize the alkaline pretreatment of orange peel as follows: reaction time (X_1); 0–40 min, KOH concentration (X_2); 0–4%, and solid loading (X_3); 40–120 g/L.

The experimental results were analyzed using analysis of variance (ANOVA). Each variable and their interactions are described by applying the following quadratic equation:

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ij} X_i X_j + \Sigma\beta_{ij} X_i^2 \tag{1}$$

where Y is the predicted response, X_i and X_j are input variables that influence the response variable Y, β_0 is the offset term, β_i is the ith linear coefficient, β_{ii} is the quadratic coefficient and β_{ij} is ijth interaction coefficient [9,37].

Table 1. Variables and their levels in the central composite rotatable design for experimental conditions of pretreatment.

Variables	Unit	Symbol	Levels				
			−2	−1	0	1	2
Time	min	X_1	0	10	20	30	40
KOH concentration	%	X_2	0	1	2	3	4
Solid loading	g/L	X_3	40	60	80	100	120

2.4. Optimization of Enzymatic Hydrolysis

The enzyme cocktail was composed of 60 FPU/g-biomass cellulase, 30 CBU/g-biomass cellobiase, and 60 XNU/g-biomass xylanase. One-unit filter paper (FPU) and cellobiase (CBU) activity was defined as the amount of enzyme that releases 1 μmol glucose per minute under standard assay conditions. One-unit xylanase (XNU) activity was defined as the amount of enzyme that releases 1 μmol xylose per minute under standard assay conditions. The activities of Celluclast® 1.5L, C-tec 2 and xylanase were determined to be 120 FPU/mL, 200 CBU/mL and 3.56 XNU/mg, respectively. The enzymatic reaction was carried out in a 50 mL conical tube by shaking at 180 rpm at 50 °C up to the maximum yield. The enzymatic digestibility (ED) was calculated using the following equation:

Enzymatic digestibility (ED, %) = (weight of glucose/(weight of glucan × 1.1)) × 100   (2)

where 1.1 is the conversion factor of glucan to glucose.

2.5. Analytical Methods

After enzymatic hydrolysis, the concentration of monosaccharides (glucose, xylose, mannose, galactose, and arabinose) in the liquor phase was investigated through high-performance liquid chromatography (HPLC) equipped with a refractive index detector (RID-10A, Shimadzu, Japan). The temperature of the column and RID was 50 °C. Samples with a volume of 20 μL were injected into a Shodex SUGAR SH1101 column (300 mm × 8 mm, Shodex, Japan) and eluted with 0.005 N sulfuric acid at a flow rate of 0.6 mL/min.

3. Results and Discussion

3.1. Characterization of Orange Peel

The mass contents of the orange flesh and peel were 65.7% and 34.3% (*w/w*), respectively. This result was in agreement with a recent study by Mahato et al. [18]. The average moisture content of whole oranges was 64.0% (*w/w*). The chemical composition of dried orange peel was investigated using the National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedure (LAP) procedure [38] and the results are shown in Table 2. An orange peel was composed of 25.0% cellulose (glucan), 17.0% hemicellulose (xylan: 11.3% and arabinan: 5.7%), and 58.0% (*w/w*) others. In another study, Senit et al. and Bustamante et al. calculated the composition of orange peel to be 18.9 ± 0.2% cellulose and 14.6 ± 0.2% hemicellulose, which was similar to our results [39,40]. As carbohydrates are correlated with fermentation, enzymatic hydrolysis was performed to separate monosaccharides for microbial growth using complex structures such as cellulose and hemicellulose [41,42]. Enzymatic hydrolysis using the non-pretreatment group resulted in approximately 25.0% glucan content (GC) and 36.0% ED. Thus, pretreatment was found to be necessary to obtain a high yield of enzymatic hydrolysis. In this study, the thermal–alkaline pretreatment was performed to reduce the reaction time and confirm the advantage of simultaneous pretreatments to improve the hydrolysis of biomass.

Table 2. The chemical composition of dried orange peel.

Compounds	Content %, *w/w* Dry Solid
Cellulose	25.0
Hemicellulose	17.0
Xylan	11.3
Arabinan	5.7

3.2. Optimization of Thermal–Alkaline Pretreatment by RSM

Thermal–alkaline pretreatment is suitable for the hydrolysis of biomass that has a cellulose and hemicellulose-linked structure. In this study, KOH was as an alkaline solvent because of its low cost and reduced environmental burden [43]. In order to obtain maximum glucose, the variables of the thermal–alkaline pretreatment were determined according to a previous study [44]. Furthermore, the experimental responses were investigated for GC and ED. Each response was calculated using orange peel hydrolysate.

Furthermore, the CCD of RSM was performed to investigate the correlation between the thermal–alkaline pretreatment and the three variables (X_1: time, X_2: KOH concentration and X_3: solid loading) of five levels (time: 0, 10, 20, 30 and 40 min; KOH concentration: 0%, 1%, 2%, 3% and 4%; solid loading: 40, 60, 80, 100 and 120 g/L) [45]. A KOH concentration of 0% indicates pretreatment with DW and a reaction time of 0 min means a control group without thermal–alkaline pretreatment. Table 3 shows the 20 designed experiments and its response. The range of each experimental data was, as follows: GC: 24.8–33.2% and ED: 29.6–95.1%.

Table 3. Central composite design (CCD), experimental and estimated data for five-level-three-factor response surface analysis.

Std	Coded Value			Glucan Content (%)	Enzymatic Digestibility (%)
	X_1	X_2	X_3		
1	−1	−1	−1	28.3	40.3
2	1	−1	−1	32.4	48.6
3	−1	1	−1	27.1	91.0
4	1	1	−1	32.8	87.8
5	−1	−1	1	29.7	29.6
6	1	−1	1	29.9	35.8
7	−1	1	1	25.1	86.5
8	1	1	1	28.8	80.1
9	−2	0	0	25.0	36.0
10	2	0	0	29.6	73.7
11	0	−2	0	33.2	31.5
12	0	2	0	28.0	95.1
13	0	0	−2	33.1	65.4
14	0	0	2	29.5	46.1
15	0	0	0	28.1	75.0
16	0	0	0	27.3	78.7
17	0	0	0	28.2	64.8
18	0	0	0	24.8	72.5
19	0	0	0	25.2	79.7
20	0	0	0	25.8	85.0

The effects of variables on GC and ED were investigated using three-dimensional (3D) response surfaces according to the established regression model (Figures 1 and 2). Figure 1 indicates the effects of the variables on GC with the 3D response surfaces. The effects of time and KOH concentration on the GC is shown in Figure 1a. The minimum amount of GC at 0 min and 3% KOH was 24.8%. When the reaction time increased, the GC also increased regardless of the KOH concentration. Furthermore, when the reaction time was reduced, lower KOH concentration led to higher GC. Figure 1b confirms the effects of time and solid loading on the GC. The GC was the lowest at 80 g/L solid loading at 0 min;

additionally, GC increased with time. The effect of KOH concentration and solid loading on GC is shown in Figure 1c. The lowest GC was obtained at 2.8% KOH concentration with 90 g/L of solid loading, and a higher GC with KOH concentration and a decrease in solid loading were observed.
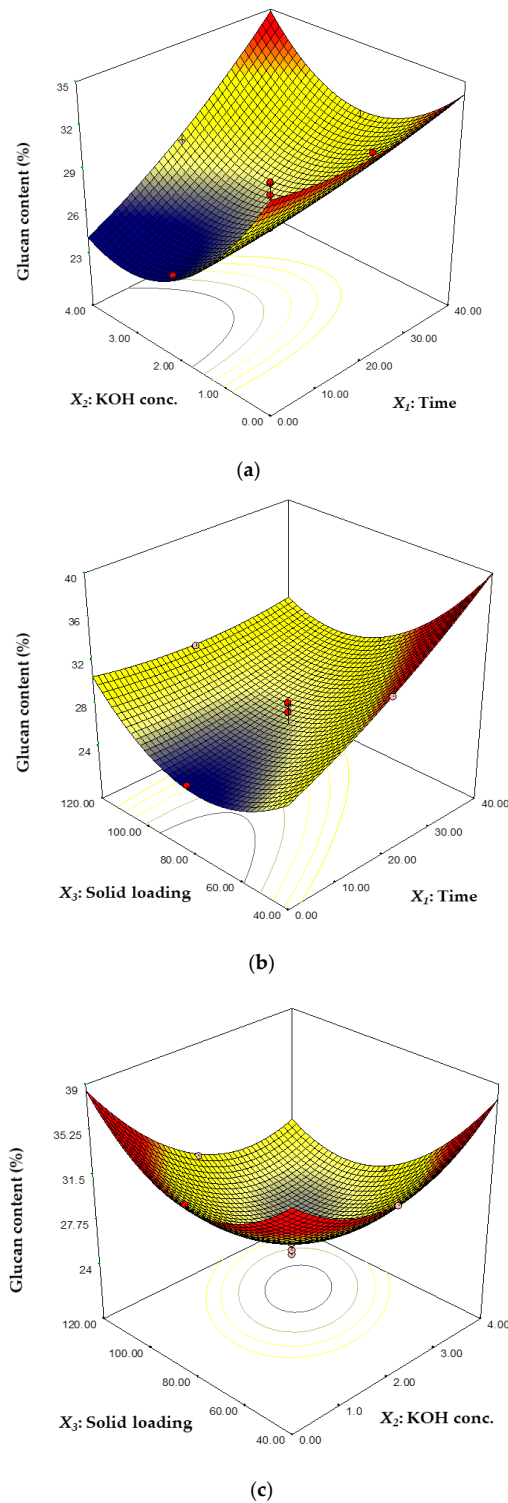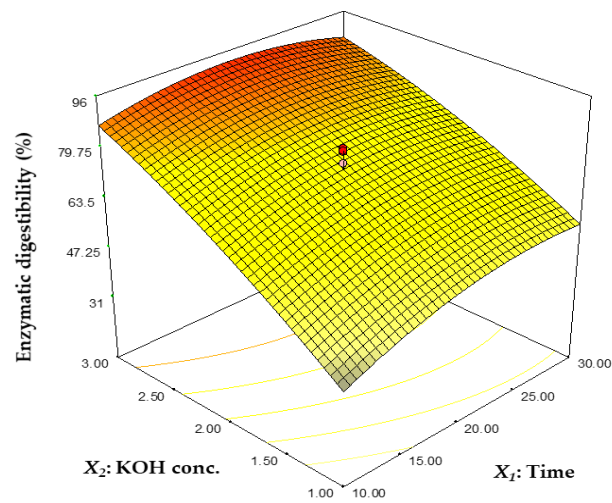

(**a**)

(**b**)

(**c**)

Figure 1. Three-dimensional (3D) response surfaces indicating the effects of thermal–alkaline pretreatment variables. The effects of X_1 and X_2 on glucan content (GC) (**a**); the effects of X_1 and X_3 on GC (**b**); the effects of X_2 and X_3 on GC (**c**). (X_1: time, X_2: KOH concentration and X_3: solid loading).
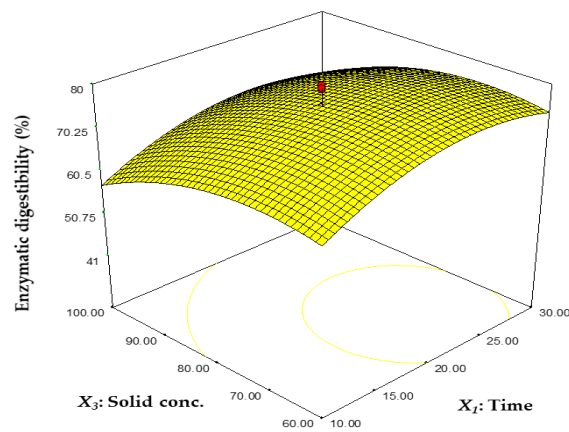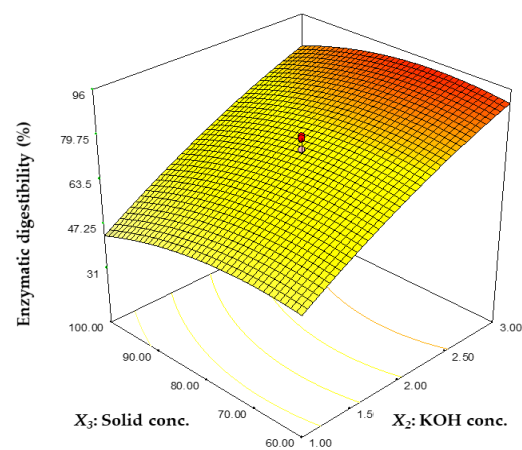
(**a**)

(**b**)

(**c**)

Figure 2. Three-dimensional (3D) response surfaces indicating the effects of thermal–alkaline pre-treatment variables. The effects of X_1 and X_2 on enzymatic digestibility (ED) (**a**); the effects of X_1 and X_3 on ED (**b**); the effects of X_2 and X_3 on ED (**c**). (X_1: time, X_2: KOH concentration and X_3: solid loading).

Figure 2 shows the effects of variables on ED using the 3D response surfaces. The effect of time and KOH concentration on ED is shown in Figure 2a. ED tended to decrease with a decrease in KOH concentration and time. Additionally, ED was high, regardless of the KOH concentration. The effect of KOH concentration and solid loading on ED is shown in Figure 2b. The ED was similar, irrespective of the time and solid loading. According to the Figure 2c, KOH concentration and solid loading are affected by ED. In general, regardless of the KOH concentration and solid loading, it showed a similar level in the 3D response surface. In particular, ED increased with increasing KOH concentration and decreasing solid loading. In other words, the higher exposure of the biomass containing cellulose, the higher the ED [46].

The CCD results were expressed as the following second-order polynomial equation by applying a quadratic regression analysis of the experimental data.

$$Y_{GC} = 26.60 + 1.42X_1 - 1.07X_2 - 0.90X_3 + 0.62X_1X_2 - 0.73X_1X_3 - 0.62X_2X_3 + 0.22X_1{}^2 + 1.04X_2{}^2 + 1.22X_3{}^2 \quad (3)$$

$$Y_{ED} = 76.10 + 3.83\,X_1 + 19.58\,X_2 - 4.33\,X_3 - 3.63\,X_1X_2 - 0.043\,X_1X_3 + 0.77\,X_2X_3 - 5.45\,X_1{}^2 - 3.09\,X_2{}^2 - 4.97\,X_3{}^2 \quad (4)$$

where Y_{GC} is the GC (%) and Y_{ED} is the ED (%) value. X_1, X_2 and X_3 are the independent variables of time, KOH concentration and solid loading, respectively. The results of ANOVA for the response surface quadratic model are shown in Tables 4 and 5.

Table 4. Analysis of variance (ANOVA) for response surface quadratic model on GC.

Source	Sum of Squares	Degree of Freedom	Mean Squares	F-Value	*p*-Value
Model	128.78	9	14.31	10.24	0.0006
X_1	32.45	1	32.45	23.22	0.0007
X_2	18.30	1	18.30	13.09	0.0047
X_3	12.98	1	12.98	9.29	0.0123
X_1X_2	3.10	1	3.10	2.22	0.1674
X_1X_3	4.31	1	4.31	3.08	0.1097
X_2X_3	3.12	1	3.12	2.23	0.1661
$X_1{}^2$	1.23	1	1.23	0.88	0.3709
$X_2{}^2$	27.35	1	27.35	19.57	0.0013
$X_3{}^2$	37.59	1	37.59	26.89	0.0004
Residual	13.98	10	1.40		
Lack of fit	3.04	5	0.61	0.28	0.9071
Pure error	10.94	5	2.19		
Total	142.76	19			

Coefficients of variation (CV): 4.13%, Coefficient of determination (R^2): 0.9021, Adjusted R^2: 0.8140, Adequate precision: 10.355.

The F-value is an indicator of the model's significance [47]. The F-values of each model were 10.24 and 21.58, respectively. To verify the significance of the model terms, the p-value of the model should be less than 0.05 [48,49]. The p-values of each model were 0.0006 and <0.0001. In this experiment, both models were considered significant models. In GC, X_1, X_2, X_3, $X_2{}^2$ and $X_3{}^2$ were significant model terms and in ED, X_1, X_2, X_3, $X_1{}^2$, $X_2{}^2$ and $X_3{}^2$ were significant model terms. The p-values of the lack-of-fit were 0.9071 and 0.0562, respectively, and each was not significant ($p > 0.05$) relative to the pure error. This shows that the quadratic model is statistically suitable for predicting responses [50]. The coefficients of variation (CV) were determined as 4.13% and 9.75% for each model. A CV lower than 10% indicates that the variation in the experimental value is within a reasonable range [51]. Meanwhile, the coefficient of determination (R^2) can be adopted to explain the reliability of the model term; the adjusted R^2 is altered by the degree of freedom [52,53]. When R^2 is greater than 0.9, it indicates high reliability. Moreover, the differences between them should be lower than 0.2 [54]. In each model, R^2 was 0.9021 and 0.9515, and the adjusted R^2 was 0.8140 and 0.9078, respectively, and the differences between them were not higher than 0.2. Finally, adequate precision refers to the signal-to-noise ratio which is a

measure of the effectiveness of the model [47]. In this case, each model can be determined as a good model with 10.355 and 17.393 of adequate precision, respectively.

Table 5. ANOVA for response surface quadratic model on ED.

Source	Sum of Squares	Degree of Freedom	Mean Squares	F-Value	*p*-Value
Model	7945.40	9	882.82	21.58	<0.0001
X_1	235.16	1	235.16	5.80	0.0368
X_2	6131.58	1	6131.58	151.25	<0.0001
X_3	299.50	1	299.50	7.39	0.0216
X_1X_2	105.44	1	105.44	2.60	0.1379
X_1X_3	0.015	1	0.015	3.594E-004	0.9852
X_2X_3	4.77	1	4.77	0.12	0.7387
X_1^2	746.02	1	746.02	18.40	0.0016
X_2^2	240.08	1	240.08	5.92	0.0352
X_3^2	619.99	1	619.99	15.29	0.0029
Residual	405.39	10	40.54		
Lack of fit	334.87	5	66.97	4.75	0.0562
Pure error	70.52	5	14.10		
Total	8350.79	19			

Coefficients of variation (CV): 9.75%, Coefficient of determination (R^2): 0.9515, Adjusted R^2: 0.9078, Adequate precision: 17.393.

The optimized conditions for the thermal–alkaline pretreatment of orange peel were designed within the experimental range of GC and ED and were determined using the regression model Equations (3)–(4) (Table 6). The optimal conditions for the thermal–alkaline pretreatment were reported as X_1: 30 min, X_2: 3% and X_3: 60.1 g/L; the predicted responses of the GC and ED were 32.3% and 86.0%, respectively. Thermal–alkaline pretreatment was performed under the optimal conditions to verify the model and the GC and ED were 32.8% and 87.8%, respectively. The verification of this model had a high level of accuracy (>98%). This indicates that the regression models can be used to optimize the orange peel pretreatment.

Table 6. Numerical optimization and validation of pretreatment based on the regression models.

Parameters	Coded Factor Levels	Actual Factor Levels
time	1.0	30 min
KOH concentration	1.0	3%
solid loading	−1.0	60.1 g/L
Response	**Predicted**	**Actual**
glucan content (%)	32.3	32.8
enzymatic digestibility (%)	86.0	87.8

3.3. Selection of Enzyme Concentration

In the fermentation process, carbon sources from organic wastes are preferred over chemical substrates (pure glucose) due to their lower costs [5–8,14]. In this study, optimal conditions for the enzymatic hydrolysis of biomass, which can be used as a carbon source, were investigated to hydrolyze the substrates in high yields. To determine the effects of thermal–alkaline pretreatment on orange peel, pretreated (experimental group) and non-pretreated (control group) were used for enzymatic hydrolysis. Three types of enzyme (cellulase, cellobiase and xylanase) were combined, and 60 FPU, 30 CBU, and 60 XNU based on g-biomass as 100%. To suggest an economic and scalable approach, the enzyme cocktail was prepared at different concentration, namely 50%, 25% and 12.5%; moreover, various loadings on enzymatic digestibility were analyzed. Consequently, both the experimental and control groups showed that ED increased proportionally with increasing enzyme concentrations (Figure 3). The thermal–alkaline pretreatment led to a maximum ED approximately 86.99%, which was 3.7-fold higher than the control group. Additionally,

the concentrations of enzyme cocktails were at 50% and 100% for 48 h. When the enzyme concentrations were 12.5% and 25%, ED in the treatment group was 3.2-fold higher than the control group by approximately 62.2% and 75.9%, respectively. As a result, keeping cost and time under consideration, an appropriate enzymatic hydrolysis process was found that was above 48 h of reaction time and 50% concentration of enzyme cocktails. This demonstrates that the ED of orange peel increases after the thermal–alkaline pretreatment.
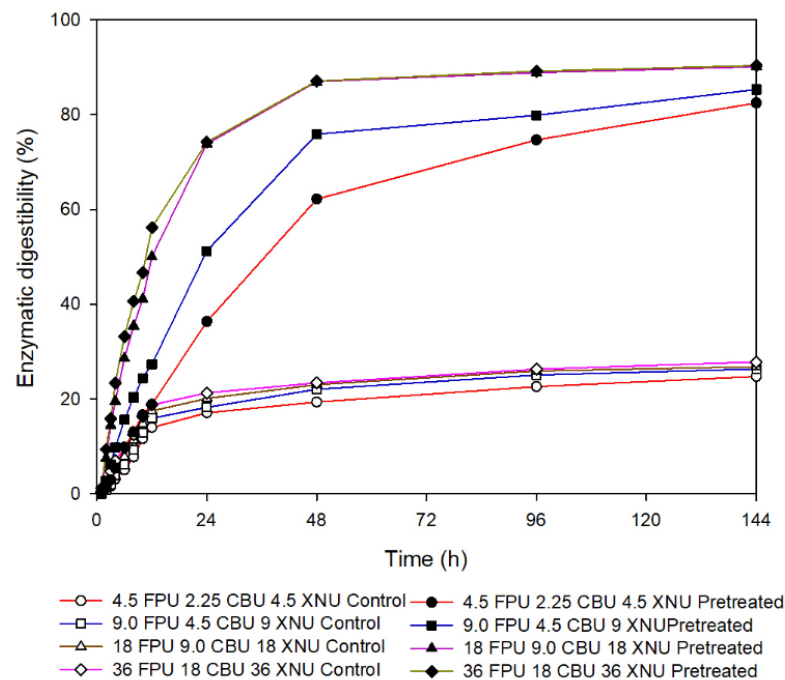

Figure 3. Enzymatic hydrolysis of orange peel. Filled symbols represent pretreated group and not filled symbols represent control group (non-pretreatment).

3.4. Summary of Pretreatment of Orange Peel

Numerous studies have focused on the effect of pretreatment on improved sugar content (especially glucose) by enzymatic hydrolysis [55–60]. Orange peels are rich in carbohydrate, which can serve as substrates in the fermentation process. Pretreatment is required to increase the accessibility of enzymes for the degradation of carbohydrates into the monosaccharides. Table 7 summarizes the various pretreatment types and enzymatic hydrolysis and compares the glucose content before and after pretreatment. Among these, popping pretreatment and steam explosion have been shown to be effective methods for high glucose yield after enzymatic hydrolysis. They can easily break the sugar complex in biomass. However, they are not appropriate for a scale-up process due to high capital cost and pressure [61,62]. Diluted acid pretreatment helps in easy sugar conversion and it reduces the damage to equipment by corrosion; however, it has a longer reaction time [63]. Other pretreatments, such as the Soxhlet and Soxhlet-liquid (two-stage) pretreatment, are time-consuming, which could be a bottleneck. Santi et al. investigated two types of pretreatment and observed that both led to low glucose content from pretreated biomass [60]. However, in this study, 1000 g of pretreated orange peel led to the recovery of approximately 316.7 g glucose after the enzymatic hydrolysis. Unlike other pretreatments, this is advantageous because of its high yields and appropriate time. Therefore, this study provides evidence of the possibility of thermal–alkaline pretreatment of orange peel for a biorefinery system.

Table 7. Summary of the increase in glucose yield based on pretreatments and enzymatic hydrolysis.

Feedstock		Pretreatment			Enzymatic Hydrolysis	Glucose Yield		Ref.
Peel	Type	Temp	Time	S/L	Enzyme Loading	Bef.	Aft.	
		(°C)	(min)	ratio	(Unit per g-Biomass)	(g per kg-Biomass)		
Mandarin	Popping	150	10	-	G (5.2 IU), P (1200 IU), X (13.25 XNU)	248	320	[55]
Lemon	Steam explosion	160	5	1:2	C (0.248 FPU), G (1190 IU), P (5.46 IU)	-	236.4	[56]
Mandarin	Steam explosion	160	5	1:2	C (0.248 FPU), G (1190 IU), P (5.46 IU)	-	153.4	[57]
Musambi	Dilute acid (0.25% H_2SO_4)	121	60	1:4.9	C (80 IU), G (100 IU), P (120 IU)	258	386	[58]
Mandarin	Soxhlet (Diethyl ether)	45	300	1:13.3	C (27.2 mg), P (18.4 mg)	155	175.2	[59]
Orange	Direct steam injection (0.5% H_2SO_4)	180	2.5	1:6.7	G (1.57 FPU)	-	34.9	[60]
Orange	Dilute acid (0.5% H_2SO_4)	130	60	1:6.7	G (1.57 FPU)	-	94.8	[60]
Orange	Thermal–alkaline	121	30	1:16.7	C (30 FPU), G (15 CBU), X (30 XNU)	66.4	316.7	This study

C: cellulase. G: cellobiase. X: xylanase. P: pectinase. IU: international units.

4. Conclusions

Orange peel, which has high potential to be a biomass feedstock in the fermentation process, needs to be pretreated to improve sugar recovery. Thermal–alkaline pretreatment was performed to improve the enzyme accessibility. The effects of the pretreatment were investigated based on two responses, GC and ED, to obtain a significant model. Optimal conditions were determined (3% KOH with solid loading of 60.1 g/L for 30 min) using a statistical method for an economical and scalable approach. In the thermal–alkaline pretreatment, GC and ED were found to be 32.8% and 87.8%, respectively. After the pretreatment, the solid fraction was used for enzymatic hydrolysis. In addition, enzyme cocktails were prepared in various concentrations to suggest an economical enzyme loading. As a result, ED was achieved at about 87% using 50% of the enzyme cocktail (30 FPU, 15 CBU, and 30 XNU based on g-biomass) for 48 h. In particular, ED of the treatment group was approximately 3.7-fold higher than the control group (non-pretreatment). Therefore, this study could offer a new scalable approach to move closer toward sustainable development.

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