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Optimizing the Conversion of Food Waste to Sugars using Fungal Enzymes

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Abstract

Food waste (FW) generally has high starch content and is rich in nutritional compounds, including lipids, proteins and acids. It is therefore potentially a renewable resource and its utilization for value-added product development is gaining interest. In this study, FW from a cafeteria was used as sole substrate for glucose production, and the fermentation conditions for optimum glucose yield were firstly optimized using response surface methodology. It was found that glucose yield was significantly affected by α-amylase loading, solid loading and temperature. The optimal conditions were found to be an α-amylase loading of 12.15 U/g FW, a solid loading of 22.4% and a culture temperature of 83.8°C for 90 min, which resulted in a maximum glucose yield of 217 mg/g. Secondly, in order to increase the final glucose concentration, an *in situ* produced fungal mash rich in glucoamylase was obtained from *Aspergillus awamori* which resulted in a glucose concentration of 99.1 g/L. When a fungal mash rich in cellulase obtained from *Trichoderma reesei* was combined with glucoamylase, a maximum of 140 g/L of glucose was obtained. This study showed that FW is a suitable substrate for saccharification with high conversion yield, indicating the potential utilization of food wastes for value-added chemicals production.

Keywords: *Food Waste; Glucose; Saccharification; amylase, Enzymatic Pretreatment*

Introduction

Increasing world population and imbalanced utilization of resources has triggered depletion of fuels, chemicals and food, and this has resulted in global warming, hunger and waste accumulation which has stressed global economic and environmental systems [1]. According to FAO [2], one third of food produced for human consumption (nearly 1.3 billion tones) is lost or wasted globally throughout the food supply chain, and it is increasing dramatically while almost 1 billion people worldwide are classified as starving. Every tone of food waste can potentially generate 4.5 ton of CO_2 emissions [3]. Currently, the majority of food waste is incinerated with other combustible municipal wastes for heat or energy production, while residual ash is then disposed of in landfills. However, incineration is an expensive waste conversion technique, and can cause severe air pollution [4]. From an environmental viewpoint, there is an urgent need for appropriate management of food waste. Due to its chemical complexity, high moisture content, putrescibility and nutrient rich composition, food waste should be treated as a useful resource for higher value products, such as fuels and chemicals through fermentation [5-12].

Saccharification is the most important step for the bioconversion of food waste into value-added products. The use of low enzyme loadings is a prerequisite for the development of a cost-efficient process. Therefore, the enzymatic saccharification process of specific food waste should be optimized. Although optimization of enzymatic hydrolysis has been reported using the one factor-at-a-time optimization approach, statistical methods for optimization are gaining growing interest and application as they have proved to be cost and time saving. Recently, several statistical experimental design methods have been employed for optimizing food waste saccharification, particularly for simultaneous saccharification and fermentation for ethanol production [13,14]. Among the optimization methods used, central composite design using response surface methodology (RSM) is a method suitable for identifying the effects of individual variables and

for seeking the optimal conditions for a multivariable system efficiently. This approach reduces the number of experiments, improves statistical interpretation possibilities and reveals possible interactions among parameters. In the present work, RSM was employed to optimize selected parameters of enzymatic hydrolysis of cafeteria food waste with α-amylase and glucoamylase for improving glucose yield. Experiments were carried out under different operational conditions defined by four independent variables (substrate loading, α-amylase/solid ratio, pretreatment temperature and duration). The role of each variable, their interactions and statistical analysis to obtain predicted yields of glucose were explained by applying a third-order cubic model. Finally, the performance of commercial enzymes was compared to fungal enzymes obtained from *Aspergillus awamori* and *Trichoderma reesei.*

Materials and Methods

Food waste characterization

The food waste used in this study was collected from a local cafeteria at Nanyang Technological University (Singapore). The food waste was ground and homogenized in a blender and stored at -20°C pending use in experiments.

Commercial enzymes

Alpha-amylase and glucoamylase used in the experiments were supplied by Genencor, Danisco Singapore Pte Ltd (Singapore). The optimal pH ranges for α-amylase and glucoamylase were 5.0 - 5.8 and 4.2 - 4.8, respectively. Amylase activity was assayed as described by Bernfeld [15]. One unit (1 U) of α-amylase activity was defined as the amount of enzyme releasing 1 μmol glucose equivalent per minute under the assay conditions.

Commercial enzymes pretreatments

Enzymatic pretreatments were conducted in 50 ml Duran bottles placed in a water bath. The suspensions were prepared by mixing the desired amounts of the prepared food waste, 100 mM sodium acetate buffer (pH 5) and α-amylase. Samples were then taken at several time intervals. After the liquefaction step using α-amylase, glucoamylase pretreatment was conducted at a fixed enzyme loading ratio of 5 U/g food waste for 2 hours.

Fungal strains and fungal enzymes production

In a previous study [16], it was found that bakery wastes, particularly waste cake, were a good substrate for glucoamylase (GA) production. In this study, *A. awamori* obtained from ABM Chemicals Ltd (Cheshire, England) was used to produce GA from waste cake through Solid State Fermentation (SSF). The waste cake was first ground to a particle size of 1.2 to 2.0 mm, sieved and then stored at -20°C for further experiments. Prior to solid state fermentation, the moisture content was adjusted to 70% (wb) with 0.1 M phosphate buffer (pH 7.9). After sterilization by autoclaving at 120°C for 20 minutes, the flasks were cooled down, and then inoculated with *A. awamori* to obtain a spore concentration of 10^6/g substrate, and the contents were mixed thoroughly with a sterile spatula. Ten grams of the mixture was distributed into several identical Petri dishes, and incubated at 30°C for 6 days under stationary conditions. The GA activity of the fungal mash harvested from two identical Petri dishes, was found to be 113.7 ± 5.2 U/gram dry solids. The fungal mash, i.e. the GA-rich fermented solids were obtained at the end of the fermentation, and it was directly used to hydrolyze mixed FW without further separation of produced enzymes.

FW contains carbohydrates other than starch like cellulose and hemicellulose. Therefore, the addition of cellulases and hemicellulases should further improve the final glucose concentration. For this reason, another fungus (*Trichoderma reesei*, ABM Chemicals Ltd, Cheshire, England) was used in solid state fermentation following the same procedure to produce a crude fungal mash rich in cellulases.

Analytical methods

Moisture and ash contents were determined according to analytical gravimetric methods [17]. Crude protein content was determined using HR Test'n tube TN kit (HACH, US) and calculated according to the Kjeldahl method with a conversion factor of 6.25. Starch content was determined using Megazyme's TN kit (Bray, Ireland). The lipid content was determined by hexane/isopropanol (3:2) method [18].

The glucose concentration was determined with Optium Xceed blood glucose monitor (Abbott Diabetes Care, Oxon, UK) [19]. Glucoamylase (GA) activity was determined with 2% (w/v) of soluble starch (Sigma) as substrate at 60°C and pH 4.8. One unit (1 U) of GA activity was defined as the amount of enzyme releasing 1 micromole glucose equivalent per minute under the assay conditions. Cellulase activity was determined using the filter paper assay as described in IUPAC procedures, and expressed in filter paper units (FPU) [20]. All the analytical assays were conducted in triplicate. To determine the saccharification degree, a conversion factor of starch to glucose of 1.11 was used [21].

Experimental Design for commercial enzymes Hydrolysis

A 2^4 full factorial design was used in the optimization of enzymatic hydrolysis of cafeteria food waste. Alpha-amylase loadings (X_1, U/g FW), substrate loadings (X_2, % in w/w), time (X_3, hour) and temperature (X_4, °C) were chosen as independent input variables. The glucose yield (mg/g FW) was used as a dependent output variable. A total of 30 experiments that included 16 cube points (runs 1 - 16), 8 star points (runs 17 - 24), and 6 replicas of the central point (runs 25 - 30) were performed to fit a third order polynomial model. The values of test variables at different temperature (42.5, 60, 77.5, 95, 112.5°C), solids (5-70%), enzyme loadings (0-14.75 U/g FW) and duration (0 - 4.25 hours) and their interaction according to central composite design (CCD) are shown in Table 2. The ranges of variables used in this work were selected based on literature and manufacturer's recommendations. For instance, 10 - 24 U/g dry solid is the recommended dosage range for starch hydrolysis, whereas 0.04% - 0.08% (w/w) dry solids is the most commonly used range for substrate loadings.

The central composite design experiments were carried out in 100 mL Duran bottles placed in a water bath. The fermentation media were prepared by mixing the desired amounts of food waste and buffer. Pretreatment experiments were started by α-amylase addition at desired concentrations. Samples were taken at different time intervals. After a certain period of liquefaction, a fixed dosage of glucoamylase (5 U/g FW) was added to the α-amylase pretreated food waste suspensions for saccharification.

Statistical Analysis

The data obtained from the central composite design experiments were analyzed using Design Expert (Stat-Ease Inc., Minneapolis, USA) (Version 8.0.7.1) software, and response surface curves, corresponding contour plots, regression coefficients and F values were obtained. Analysis of variance (ANOVA) was applied for the response function. The actions and interactions of the variables were estimated by the following third-order cubic equation:

$$Y = b_0 + \sum b_i X_i + \sum b_{ij} X_{ij} + \sum b_i^2 X_i^2 + \sum ij^2 X_{ij}^2 + \sum i^2 j X_{ij}^2 + b_i^3 X_i^3 \quad (1)$$

where Y is the predicted response for glucose yield (mg/g FW); b_0 is the intercept; b_i is the coefficient for linear direct effect; b_{ij} is the coefficient for interaction effect; b_i^2 and b_i^3 are the coefficients for quadratic and cubic effects (a positive or negative significant value implies possible interaction between the medium constituents); X_i, X_{ij}, $X_{ij}^2$and X_i^3 are the independent variables. The quality of fit to the third order equation was expressed by the coefficient of determination (R^2), and its statistical significance was determined by the F-test. Variables with probability below 95% ($P > 0.05$) were regarded as not significant to the final models. Three-dimensional surface plots were drawn to illustrate the main and interactive effects of the independent variables on the dependent variables. The influence of experimental error on the central composite design was assessed with six replications at the central point of the experimental domain. Experiments were carried out in triplicate. Results were presented as the average of three independent trials. To maximize the glucose recovery from α-amylase pretreatment, numerical optimization was used for determination of the optimal levels of the four variables.

Model validation

Two sets of experiment were studied to validate the model. Enzymatic hydrolyses were conducted under the optimal conditions established from the experimental work:

(i)To obtain the highest glucose yield, α-amylase loading of 12.15 U/g FW, solids loading of 22.41%, temperature of 84°C, and hydrolysis time of 90 minutes;

(ii)To obtain high glucose yield at high solid but low enzyme loading: α-amylase loadings of 10 U/g FW, solids loading of 35%, temperature of 81°C, and hydrolysis time of 123 minutes.

All experiments were performed in triplicate and standard deviations were calculated from the mean of the triplicate analyses.

Results and Discussion

Food waste composition and characterization

Table 1 shows the composition of the food waste used in this study. Approximately 57% of the total dry matter was starch.

Moisture (%)	65.0 ± 1.2
Total Solid (%)	35.0 ± 1.2
TS/VS (%)	94.6 ± 1.2
Starch (%), db	57.1 ± 1.4
RS (%), db	1.6 ± 0.0
Protein (%), db	9.5 ± 2.2
Lipid (%), db	11.2 ± 0.5
Ash (%), db	3.8 ± 0.3

Table 1: *Composition of food waste used in this study.*

Total solid, starch, reducing sugar (RS), lipid, protein and ash contents were given in wt% on the basis of dry weight (db). Volatile solid (VS) contents were given as the %VS ratio on total solid basis.

Optimization and modeling of glucose yield from commercial enzymes hydrolysis

To optimize the saccharification and maximize the glucose production, the effects of solid and enzyme loadings, temperature and time were investigated through 30 sets of experiments as described earlier. The experimental conditions and the responses are summarized in Table 2.

A reduced cubic model was used to fit to experimental results. The regression equation (Equation 2) obtained after the analysis of variance (ANOVA) showed the dependence of the glucose concentration on substrate and enzyme loadings, time and temperature:

$$Y = +126.01 + 77.28 * X_1 + 40.49 * X_2 + 23.22 * X_3 + 15.63 * X_4 - 10.14 * X_{12} + 4.05 * X_{13} -0.082 * X_{14} - 3.41* X_{23} - 31.45 * X_{24} - 3.11 * X_{34} - 32.08 * X_{11} X_2 -24.12*X_1X_{22} \quad (2)$$

where X_1, X_2, X_3 and X_4 are independent variables representing the α-amylase loading, substrate loading, time and temperature, respectively, and Y represents the glucose yield (mg/g FW) as a response variable. The ANOVA analysis suggested that Equation 2 was significant at the 95% confidence level ($P < 0.001$) (Table 3). The model F-value of 14.99 implies that the model is significant, and there is only a 0.01% chance that a "model F-value" this large could occur due to noise. Values of "Prob>F" less than 0.05 indicate that the model terms are significant, meaning that X_1, X_2, X_3, X_4, X_{12}, X_{24}, X_{11}, X_{22}, X_{44}, X_{124}, X_{112} and X_{111} are the significant model terms (Table 3). Whereas, the values greater than 0.1 indicate that the model terms are not significant. The coefficient of determination (R^2) for the enzyme activity was calculated as 0.98, showing that the model can explain 98% of variability in the response. The parity plot (Figure 1) shows the correlation between the experimental and predicted values of the response. It represents an acceptable variation between the experimental and predicted glucose yields in the range of the operating variables. The high value of R_2 indicates that the reduced cubic equation can provide a reasonable description of the system performance in the given experimental domain. Adequate precision measures the signal to noise ratio and a ratio greater than 4 is desirable. In this study, a ratio of 16.62 was obtained, indicating that noise was negligible. Therefore, the proposed model can be used to navigate the design space.

Run	X_1 [a]	X_2 [b]	X_3 [c]	X_4 [d]	Glucose yield (mg/g FW)	
	Actual (coded)	Actual (coded)	Actual (coded)	Actual (coded)	Experimental	Predicted
1	10 (+1)	10 (-1)	3 (+1)	60 (-1)	111.48	112.50
2	10 (+1)	50 (+1)	3 (+1)	60 (-1)	127.43	127.60
3	0.5 (-1)	50 (+1)	3 (+1)	60 (-1)	138.24	132.70
4	0.5 (-1)	10 (-1)	3 (+1)	60 (-1)	19.094	23.22
5	10 (+1)	10 (-1)	0.5 (-1)	60 (-1)	59.67	60.57
6	10 (+1)	50 (+1)	0.5 (-1)	60 (-1)	128.832	135.39
7	0.5 (-1)	10 (-1)	0.5 (-1)	60 (-1)	1.7784	0.56
8	0.5 (-1)	50 (+1)	0.5 (-1)	60 (-1)	110.496	108.26
9	0.5 (-1)	50 (+1)	0.5 (-1)	95 (+1)	81.024	77.98
10	0.5 (-1)	10 (-1)	0.5 (-1)	95 (+1)	131.415	135.26
11	10 (+1)	10 (-1)	3 (+1)	95 (+1)	189.4	195.26
12	10 (+1)	50 (+1)	0.5 (-1)	95 (+1)	112.99	114.79
13	0.5 (-1)	10 (-1)	3 (+1)	95 (+1)	133.944	138.99
14	10 (+1)	50 (+1)	3 (+1)	95 (+1)	120.673	116.76
15	0.5 (-1)	50 (+1)	3 (+1)	95 (+1)	84.7675	87.33
16	10 (1)	10 (-1)	0.5 (-1)	95 (+1)	141.898	137.40
17	14.75 (+α)	30 (0)	1.75 (0)	77.5 (0)	136.785	135.3
18	0.0 (-α)	30 (0)	1.75 (0)	77.5 (0)	132.66	134.64
19	5.25 (0)	70 (+α)	1.75 (0)	77.5 (0)	94.7575	95.33
20	5.25 (0)	5 (-α)	1.75 (0)	77.5 (0)	31.0465	30.75
21	5.25 (0)	30 (0)	4.25 (+α)	77.5 (0)	139.095	136.29
22	5.25 (0)	30 (0)	0 (-α)	77.5 (0)	79.2	79.86
23	5.25 (0)	30 (0)	1.75 (0)	112.5 (+α)	80.8725	84.11
24	5.25 (0)	30 (0)	1.75 (0)	42.5 (-α)	17.013	18.89
25	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	138.6	123.48
26	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	132.825	123.48
27	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	113.025	123.48
28	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	131.67	123.48
29	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	106.755	123.48
30	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	125.565	123.48

Table 2: *Central composite design with observed and predicted responses of glucose yields. Each row corresponds to a single experiment.*

[a]Coded values of α-amylase loadings; [b]Coded values of substrate loadings; [c]Coded values of time; [d]Coded values of temperatures.

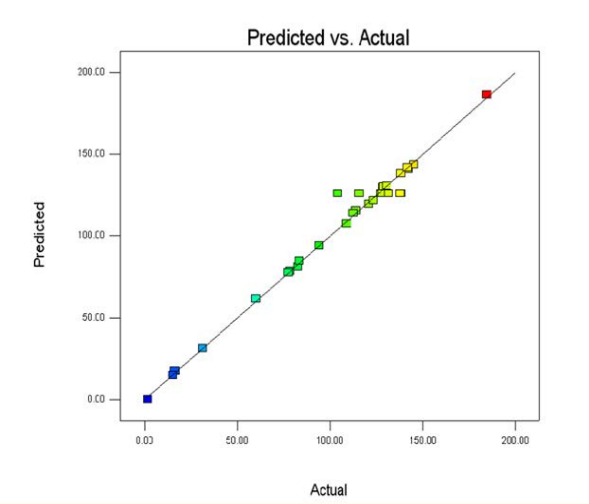


Figure 1: *The observed versus predicted glucose yields under the experimental conditions.*

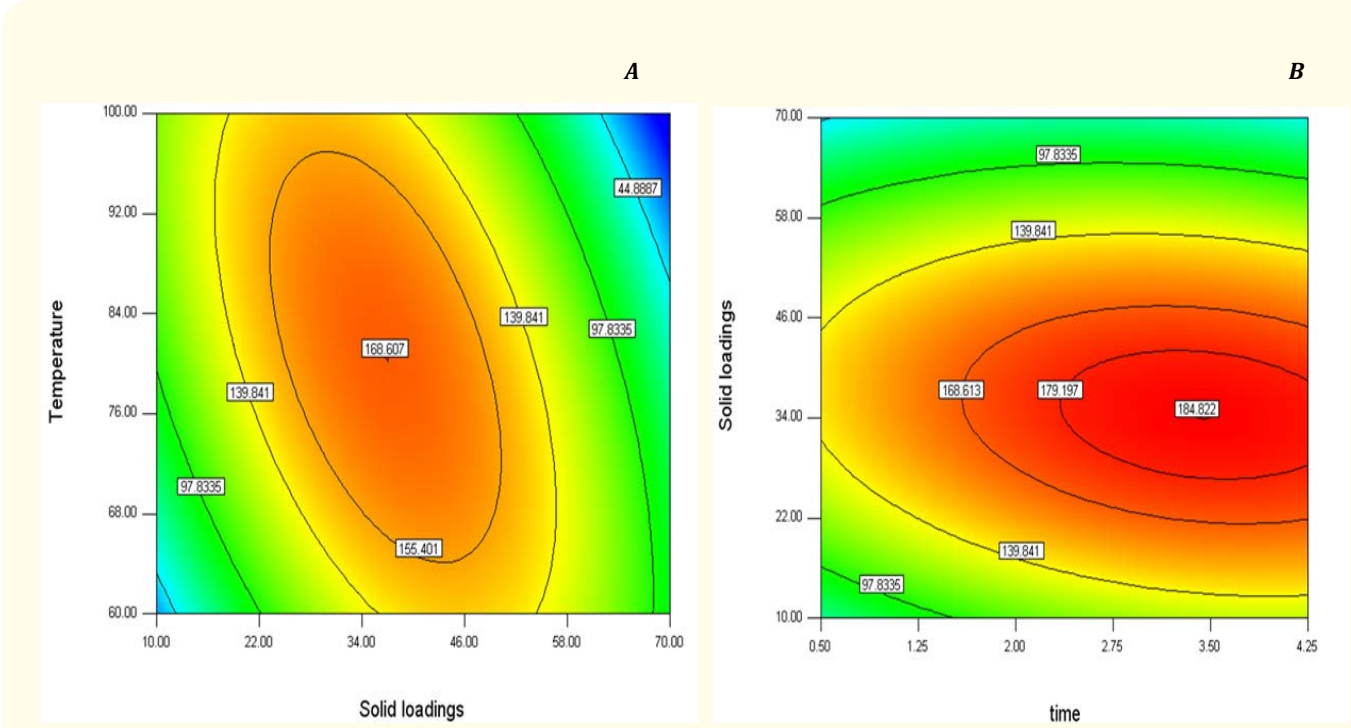
Source	Sum of Squares	DF	Mean Square	F Value	p-value Prob > F
Model	54091.08	23	2351.79	14.99	0.0014*
X_1- α-amylase loading	1646.22	1	1646.22	10.50	0.0177*
X_2- solid loading	5568.49	1	5568.49	35.50	0.0010*
X_3- time	2378.71	1	2378.71	15.17	0.0080*
X_4- temperature	1953.31	1	1953.31	12.45	0.0124*
X_{12}	1644.28	1	1644.28	10.48	0.0177*
X_{13}	261.86	1	261.86	1.67	0.2438
X_{23}	186.06	1	186.06	1.19	0.3179
X_{24}	15826.45	1	15826.45	100.91	< 0.0001*
X_{11}	4978.60	1	4978.60	31.74	0.0013*
X_{22}	7167.27	1	7167.27	45.70	0.0005*
X_{33}	669.73	1	669.73	4.27	0.0843
X_{44}	9509.37	1	9509.37	60.63	0.0002*
X_{124}	1487.35	1	1487.35	9.48	0.0217*
X_{112}	2883.95	1	2883.95	18.39	0.0052*
X_{111}	2545.79	1	2545.79	16.23	0.0069*
Residual	941.04	6	156.84		
Lack of Fit	36.42	1	36.42	0.20	0.6724
Pure Error	904.61	5	180.92		
Corrected Total	55032.12	29			

Table 3: *ANOVA for glucose yield during enzymatic hydrolysis of FW as a function of alpha-amylase loading (X_1), solid loading (X_2), time (X_3) and temperature (X_4).*

**Significant variable; FW, food waste; DF, degree of freedom; determination coefficient (R^2), 0.98; adjusted determination coefficient (R^2adj), 0.92; co-efficient of variation (CV), 11.94%; adequate precision ratio, 16.62.*

The statistical analysis in table 3 suggests that all the independent variables have a significant effect on the response (i.e. glucose yield). The positive correlation between X_1 and X_2 indicate the presence of a linear, enhancing effect on the saccharification. However, the negative correlations among X_{12}, X_{14}, X_{23} and X_{34} suggest such quadratic and linear interaction would not favor the response (glucose yield), while the positive correlation for X_{13} improve the response. On the other hand, the negative coefficients for X_{112} and X_{122} expose the existence of cubic interaction effects that decrease the glucose production.

The response surface plots are presented in Figure 2 as a function of two variables at time t, while keeping the third and fourth variables constant (zero, coded units). It is apparent from Figure 2A that a glucose yield of 168.6 mg/g FW was obtained at an enzyme loading of 7.25 U/g FW within 105 minutes. At 80°C, the increasing solid loading from 20 to 35% resulted in 18% increase in the glucose yield (Figure 2A). However, when the substrate loading was further increased to 50% (w/w), the glucose yield decreased from 168.6 to 154 mg/g FW. This might be due to the increased density and decreased water content of the suspension which prevented the action of α-amylase on FW. The increase in glucose yield was 14.4% (increased from 139.8 to 160 mg/g FW) with an increase in the residence time from 30 to 90 minutes, while it was 7.5% (from 160 to 172 mg/g FW) from 90 to 150 minutes (Figure 2B) at 80°C using 7.25 U/g FW α-amylase loading. With less than 25% solid loading the glucose yield did not improve more than 168 mg/g FW with time, while it was similar using more than 45% solid loading using 7.25 U/g FW α-amylase loading. Therefore, the solid loadings should be between 25 - 45% to improve the hydrolysis. The α-amylase loading was also dependent on time and temperature (Figure 2C and 2D). The optimal α-amylase loading range should be between 7.3 and 13.2 U/g FW, otherwise more than 25% of the starch would remain intact. Using 35% solid loading, the highest glucose yield (203 mg/g FW) was obtained using 10.7 U/g FW α-amylase at 83.8°C within 105 minutes. The increase in the glucose yield was negligible when the hydrolysis was extended for more than 3 hours (Figure 2D). Using the optimum temperature (83.8°C), the highest glucose yield (217 mg/g FW) was expected using 22.4% solid loadings for 90 minutes (2E).



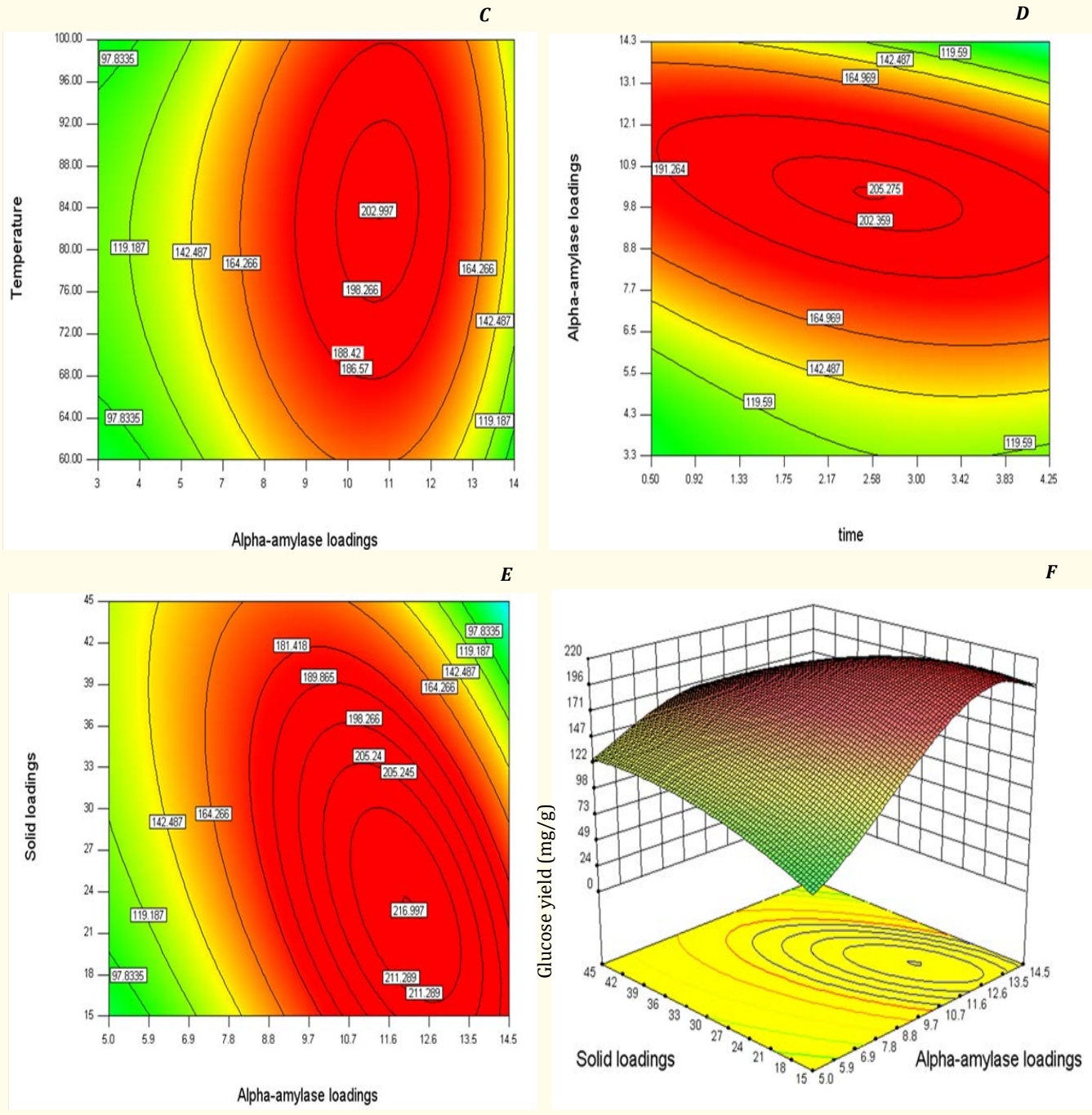


Figure 2: *Response surface plots, described by Eq. (2), representing the effect of solids loadings and temperature using 7.25 U/g FW α-amylase for 1.75h (2A); solids loadings and time using 7.25 U/g FW α-amylase at 80°C, (2B); α-amylase loadings and temperature using 35% solid loadings for 1.75h (2C); α-amylase loadings and time using 35% solid loadings at 83.8°C (2D), and solid loadings and α-amylase loadings at 83.8°C for 1.5h (2E and 2F) on glucose yield from domestic FW.*

Model validation

To validate the model developed in this work, the experiments were run under the optimal conditions for enzymatic hydrolysis obtained from the model prediction (i.e. enzyme loading of 12.15 U/g FW, FW loading of 22.4%, temperature of 84°C and fermentation time of 1.5h). Under these experimental conditions, a glucose yield of 217.5 mg/g FW was obtained, which was in excellent agreement with the predicted value of 217 mg/g FW as shown in Figure 2E. This shows a yield increase of 43% compared to 123.48 mg/g FW on average achieved in the non-optimized controls.

Another independent run resulted in a glucose yield of 214.5 mg/g FW under the following conditions: 10 U/g FW, 35% solids loading, at 81°C for 2.05h, which validates the accuracy of the model and the potential of FW to produce value-added products after enzymatic hydrolysis.

A glucose concentration of 53 g/L with a glucose yield of 217.5 mg/g FW were obtained using a solid loading of 22.4%, while 70.4 g/L with a glucose yield of 214.5 mg/g FW was achieved using a solid loading of 35% solid loading. This means that high solid loading would result in a lower glucose yield, but a higher glucose concentration.

Comparison of commercial and fungal enzymes in the saccharification of FW

Once the right conditions to optimize the glucose yield using commercial enzymes were found, the second objective was to increase the glucose concentration for potential biorefinery application, and compare the performances of commercial and fungal enzymes obtained after solid state fermentation. In order to compare the performance, the same enzymes dosage of 10 U/g FW was applied and in order to increase the glucose concentration, a solid loading of 50%(w/v) was applied in both cases.

It can be seen in Figure 3 that a glucose concentration of 99.1 ± 7 g/L was obtained after 24 hours in the flasks supplied with the fungal mash, while 77.2 ± 6.9 g/L was reached with commercial enzymes under the same experimental conditions. It should also be noted that the initial glucose production rate with the fungal mash was significantly higher than with commercial enzymes. Given the complex composition of FW, different enzymes are required for a high-efficiency hydrolysis and saccharification, and the fungal mash contains tailor-made enzymes mixture well adapted for the hydrolysis of starch and the subsequent saccharification.

The glucose concentration that can be derived from FW depends on the carbohydrate content of FW. Kim et al. [22] studied the saccharification of different kind of FWs using an enzyme cocktail containing cellulolytic and amylolytic enzymes, and found that high reducing sugars concentration was obtained from cafeteria FW with high organic content as compared to the FWs collected from apartments and restaurants with relatively low organic contents.

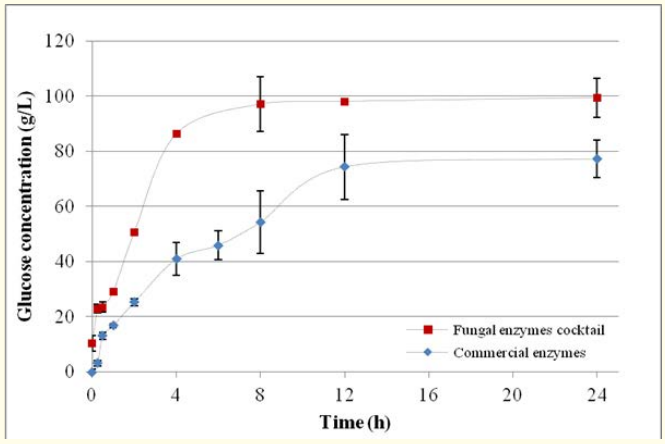


Figure 3: *Comparison of commercial enzymes and fungal enzymes (10 U/g FW) hydrolysis of food waste. Each data point is the average of triplicate measurements from duplicate experiments and the error bars represents the standard deviations.*

Cekmecelioglu and Uncu [23] developed a complex pre-treatment procedure involving multiple commercial enzymes namely α-amylase, glucoamylase, cellulase and β-glucosidase. During liquefaction, α-amylase was added (120 U/g dry substrate) to the waste and kept at 95°C for 1 hour at 100 rpm and pH 5.5. The saccharification proceeded simultaneously when adding the enzymes glucoamylase (120 U/g dry substrate), cellulase (8 FPU/g dry substrate) and β-glucosidase (50 U/g dry substrate) at 55°C. The highest glucose concentration was only 64.8 g/L achieving 70% conversion after 6 hours of enzymatic hydrolysis of FW (Table 4).

Enzyme	Vessel type	Conditions	Concentration (g RS/L)	SD	References
GA (0.16% (v/v))	500 mL flask with 100 mL working vol.	46.3°C, pH5.2, 4h, S/L:1 (v/v)	120.1	NR	Kim., *et al.* [13]
GA (170 mg/kg FW)	3L jar with 1.5 kg working vol.	60°C, 100 rpm, 6h, S/L: 2 (v/v)	67.2	0.85	Tang., *et al.* [27]
GA (120 U/g FW)	NR	35°C, pH 5.5, 60h, S/L: 2 (w/v)	85	NR	Wang., *et al.* [28]
α-amylase (NR), GA (1.2 U/g)	NR	50°C, pH 4.5-6, 24h	55	NR	Hong and Yoon [29]
Carbohydrase (8U/g), GA, cellulase	5L fermenter with working vol. of 3L	35°C, pH 4.5-6, 9h, S/L:1 (w/v)	20	0.63	Kim., *et al.* [30]
α-amylase (120 U/g ds), GA (120 U/g ds), Cellulase (8 FPU/g ds), β-glucosidase (50 U/g ds)	NR	95°C pH5.5, 100rpm, 1h and 55°C, pH5.5, 100 rpm 5h, S/L:0.2	105	NR	Uncu and Cekmecelioglu [31]

α-amylase (120 U/g ds), GA (120 U/g ds), cellulase (8 FPU/g ds), β-glucosidase (50 U/g ds)	NR	95°C pH 5.5, 100rpm, 1h and 55°C, pH 5.5 100 rpm 5h, S/L:0.1	64.8	NR	Cekmecelioglu and Uncu [32]
α-amylase (10 U/g FW), GA (120 U/g FW)	500 mL flask with 400 g working vol.	55°C, pH 4.5, 48h, S/L:1	131.4	0.93	Yan., *et al.* [33]
α-amylase (10 U/g FW), GA (5 U/g FW)	50 ml duran bottle	85°C, pH 5, 2.05h and 60°C, pH 5, 2h S/L: 0.35	70.4	0.78 - 0.82	This study
Fungal mash GA (10 U/g FW)	50 ml duran bottle	60°C, pH 5, 24h S/L: 0.5	99.1	0.85 - 0.95	This study
Fungal mash GA (7 U/g FW) + fungal mash cellulose (1 FPU/g FW)	50 ml duran bottle	60°C, pH 5, 24h S/L: 0.5	140	0.95 - 0.99	This study

Table 4: *Glucose concentrations and yields from FWs by enzymatic hydrolysis.*

GA: Glucoamylase; RS: Reducing sugar; ds: Dry substrate; S/L: solid loading (w/w); SD: Saccharification degree; NR: not reported.

This is lower than the glucose concentration of 86 g/L obtained in this study after 4 hours hydrolysis of mixed FW with fungal mash (Figure 3). Although *A. awamori* is known to be an efficient producer of glucoamylases, it can also produce hydrolytic enzymes, such as amylases, proteases, cellulases and xylanases when growing on complex substrates, such as mixed FW in SSF [24,25]. It has been reported that fermented solids obtained from the SSF of babassu cake with *A. awamori* contained considerable activities of proteases, xylanases and cellulase activities besides amylases [25]. About 90 - 95% of starch in FW was hydrolyzed by the fungal mash produced in this study. This strengthens the hypothesis that this fungal mash contained other carbohydrases such as α-glucosidases, β-amylases, β-glucanases pullulanases, cellulases, xylanases hemicellulases, besides glucoamylase. The fungal mash produced in this study required only one step whereas the commercial enzymes treatment required two steps, each optimized for a particular enzyme. The temperature required for the fungal enzymes was also lower compared to the liquefaction step using α–amylase.

Various agricultural and kitchen waste residues were assessed for their ability to support the production of cellulase by *Trichoderma reesei* in solid state fermentation (Figure 4). Different FWs such as banana peel, soybean flour, potato peels, oat meal and orange waste were used as sole substrate to produce cellulases as the highest cellulase activities were reported using these substrates in the literature. The substrates were found to be well suited for fungal growth, producing good amounts of cellulases after 96 hours. The highest cellulase activity (12.2 FPU/mL) was obtained using oat meal (Figure 4).

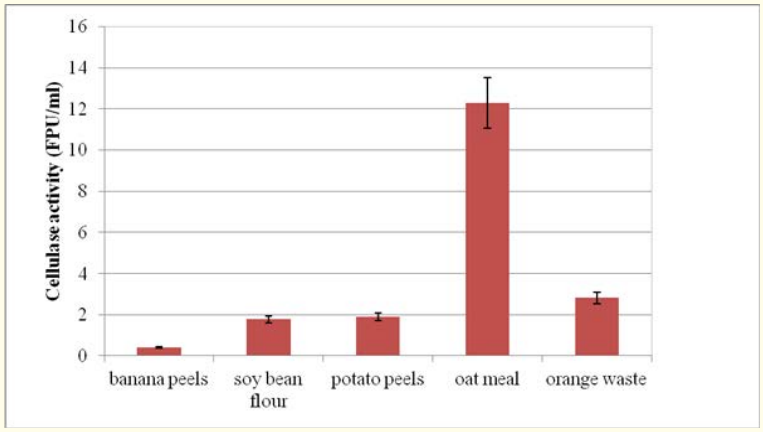


Figure 4: *Effect of different substrates on cellulase production using T. reesei using SSF at 25°C, 6 days. Data points show the averages from duplicate analyses.*

The effect of crude enzymes rich in GA and cellulase were then evaluated on the saccharification of FW. Figure 5 shows that a fungal mash rich in GA resulted in 115 g/L glucose after 24 hours hydrolysis, while only 36.5 g/L glucose was achieved using the fungal mash rich in cellulase. More significantly, the hydrolysis of complex FW was improved by the co-utilization of both enzymes cocktails together. Using 7 U/g FW GA and 1 FPU/g FW cellulase, a maximum of 140.1 g/L glucose was produced.

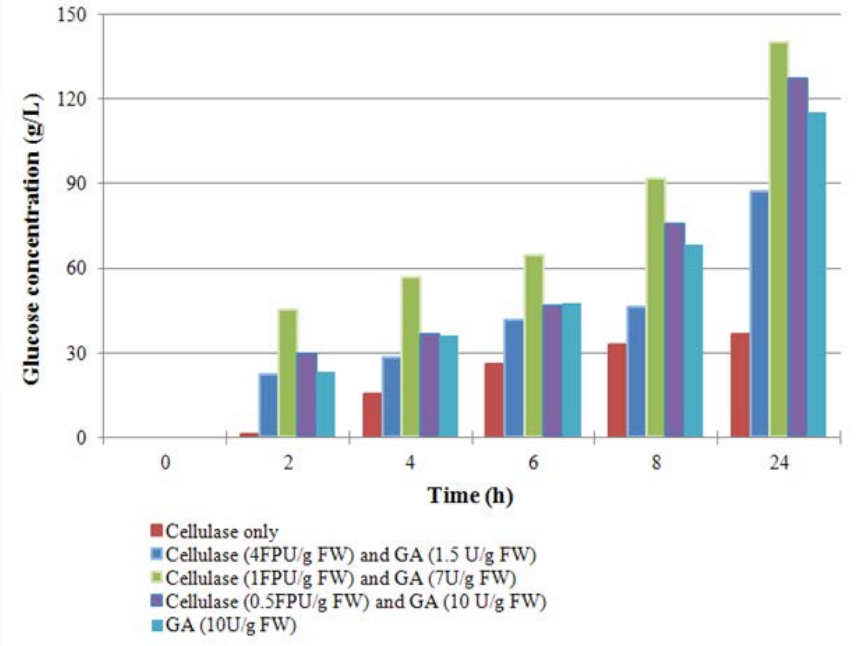


Figure 5: *Effect of GA and cellulase rich enzymes cocktails on glucose production using FW loading of 50%, at 60°C for 24 hours. Data points show the averages from duplicate analyses.*

The optimization of the hydrolysis step is very important for saving money and time. The glucose concentration obtained by fungal mash pretreatment was higher than those reported in the previous studies with commercial enzymes (Table 4). For instance, Kim., *et al.* [22] reported that the higher food waste concentration caused a faster initial saccharification and larger reducing sugar production, and after 24 hours they obtained 98 g/L reducing sugars from the hydrolysis of 200 g/L food wastes using an enzyme cocktail produced from *Trichoderma harzianum*. In the study by Pleissner., *et al.* [26], a glucose concentration of 143 g/L was obtained after 48 hours fermentation. In this study, a similar titer was obtained in a shorter time of 24 hours.

Conclusion

In this work, modeling of enzymatic hydrolysis of domestic FW with α-amylase and glucoamylase was successfully performed using central composite design and response surface methodology. The effects of solids loading, enzyme loading, time and temperature as independent variables were investigated. All of the variables showed significant impact on enzymatic hydrolysis with the solids loading being the most significant. Under the optimum enzymatic hydrolysis conditions (22.4% solid loadings, 12.15 U/g FW at 85°C for 90 minutes.), the model validation experiment produced a maximum glucose experimental yield of 217 mg/g which represents an increase of 43% over the non-optimized controls at the central points (123.48 mg/g FW on average). *In situ* enzymes were produced using *A. awamori* growing under solid state fermentation, and the fungal mash rich in glucoamylase resulted in greater glucose concentration (99.1 g/L) compared to pure commercial enzymes (70.4 g/L). When cellulases (1 FPU/g FW) was combined with glucoamylase (7 U/g FW), a maximum of 140.1 g/L glucose was obtained which represents a significant advance in the production of glucose for the subsequent fermentation to platform chemicals or biofuels.

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