ETRP PROGRESS REPORT

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GUIDELINES FOR ETRP PROGRESS/FINAL REPORT

Project Reference No.: ETRP 1201 105	**Project Completion Date:** 12/2014
Project Title: Developing Novel Biorefineries using Food Waste as Substrate	
Organization: Nanyang Technological University, NEWRI, AEBC	**Officer-in-charge:** Joycelyn Tang

Project Period (Please tick the appropriate box)

X July Progress Report for the period <*12/2012*> to <*07/2013*>

□ Final Report (Part A & B)

Part A

1. PROJECT MANAGEMENT AND EXECUTION

The project is progressing smoothly on schedule as shown below. The projected milestones and deliverables have all been achieved as detailed in Section "Results and Discussion". As of July 2013, we have completed the literature review and characterization of food wastes. The two bioreactors with essential control software will be ready soon for the next phase experiments. Meanwhile, the saccharification level of food wastes (from cafeteria) was investigated with commercial enzymes, and the conditions were also optimized. The preliminary studies of solid state enzyme production were conducted, based on which the process optimization was then carried out for improving the enzyme production.

Milestones and Deliverables	**Implementation Schedule**								
	Year 1				**Year 2**				**Remarks**
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	
Literature Review									
1: Characterisation of KW									
2: Construction of 2 HRs (2-5L)									
3: Liquid state fermentation of FW by *Aspergillus*: characterization of enzymes									
Actual Implementation									
4: Solid State fermentation of FW by *Aspergillus* characterization of enzymes									
5: Optimization of in-house enzymes production									
6: Enzymatic hydrolysis of KW using in-house enzymes cocktail produced by *Aspergillus*									
Actual Implementation									

2. RESULTS & DISCUSSION

2.1 FW Characterization

According to the literature, food waste (FW) is mainly composed of carbohydrate polymers (starch, cellulose, hemicelluloses), lignin, proteins, lipids, acids, and a smaller inorganic portion (Table 1). Hydrolysis of carbohydrate components, particularly starch, causes the breakup of glycoside bonds of polysaccharides, leading to production of oligosaccharides and/or

maltodextrins and monosaccharides, which can be further converted to valuable products through fermentation, e.g. gaseous and liquid biofuels. Total sugar and protein contents in food waste were typically in the range of 35.5-69.0% and 3.9-21.9%, respectively.

Table 1. Composition of mixed food waste.

Moisture	Total solid	Volatile solid	Total sugar	Starch	Cellulose	Lipid	Protein	Ash	
79.5	20.5	95.0	NA	NA	NA	NA	21.9	NA	Han and Shin (2004)
84.1	15.9	15.2	NA	NA	NA	NA	NA	NA	Kim et al. (2004)
80.0	20.0	93.6	NA	NA	NA	NA	NA	1.3	Kwon and Lee (2004)
85.0	15.0	88.5	NA	NA	15.5	8.5	6.9	11.5	Rao and Singh (2004)
79.1	20.9	93.2	NA	NA	NA	NA	NA	NA	Ramos et al. (2012)
75.9	24.1	NA	42.3	29.3	NA	NA	3.9	1.3	Ohkouchi and Inoue (2006)
87.1	12.9	89.5	NA	NA	NA	NA	NA	NA	Kim et al. (2008)
80.8	19.2	92.7	NA	15.6	NA	NA	NA	NA	Pan et al. (2008)
80.3	19.7	95.4	59.8	NA	1.6	15.7	21.8	1.9	Tang et al. (2008)
82.8	17.2	89.1	62.7	46.1	2.3	18.1	15.6	NA	Wang et al. (2008a)
75.2	24.8	NA	50.2	46.1	NA	18.1	15.6	2.3	Wang et al. (2008b)
85.7	14.3	98.2	42.3	28.3	NA	NA	17.8	NA	Zhang et al. (2008)
82.8	17.2	85.0	62.7	46.1	2.3	18.1	15.6	NA	Ma et al. (2009)
61.3	38.7	NA	69.0	NA	NA	6.4	4.4	1.2	Uncu and Cekmecelioglu (2011)
64.4	35.6	NA	NA	NA	NA	8.8	4.5	1.8	Cekmecelioglu and Uncu (2012)
81.7	18.3	87.5	35.5	NA	NA	24.1	14.4	NA	He et al. (2012)
81.5	18.5	94.1	55.0	24.0	16.9	14.0	16.9	5.9	Vavouraki et al. (2012)
81.9	14.3	98.2	48.3	42.3	NA	NA	17.8	NA	Zhang et al. (2013)

Total Solid, Total sugar, Starch, Cellulose, Lipid, Protein and Ash Contents were given in wt% on the basis of dry weight. Volatile solid contents were given as the %VS ratio on total solid basis.

In this study, nine different kinds of FWs were collected from supermarkets, cafeterias and houses. They were first homogenized using a kitchen blender and then stored in tightly closed food bags at -20^{o}C until further use. The compositions of the FWs were determined in triplicate, and the average values are presented in Table 2. The compositions of the FWs were all different. For example, the solid content of pastries like cake, bread and savory were higher, while higher moisture contents were found in vegetable, potato and fruits. The mixed food wastes made up from cafeterias and kitchen had a higher protein and lipid content, which might favor microbial growth during fermentation.

Table 2. Composition of different FWs.

FW (origin)	Moisture (%)	TS (%)	VS/TS (%)	Starch (%), db	RS (%), db	Protein (%), db	Lipid (%), db	Ash (%), db
Bread (Supermarket)	34.4±0.2	65.6±0.2	96.7±0.0	71.6±0.5	0.5±0.1	8.6±2.1	3.9±2.6	3.2±0.0
Cake (KG catering)	29.9±1.9	70.1±1.9	96.0±0.3	33.5±3.0	16.8±0.5	4.1±0.8	16.1±7.5	3.9±0.2
Fruits (ShengSiong)	83.8±2.2	16.2±2.2	96.6±0.6	24.8±4.5	11.7±1.5	3.5±0.4	1.0±0.2	3.4±0.6
Potato (ShengSiong)	82.4±0.7	17.6±0.7	97.2±0.7	47.6±5.5	1.2±0.1	6.9±2.2	0.2±0.0	2.7±0.5
Savory (KG catering)	37.8±0.4	62.2±0.4	96.6±0.3	45.7±2.8	0.3±0.0	2.3±1.1	22.1±0.3	3.3±0.4
Vegetables (ShengSiong)	95.2±0.6	4.8±0.6	85.7±2.0	16.4±0.1	0.0±0.0	0.5±2.2	1.5±0.1	11.3±1.3
FW1 (ShengSiong's Cafeteria)	80.3±1.1	19.7±1.1	95.2±0.4	19.0±1.3	0.7±0.0	15.4±2.4	19.4±0.1	4.7±0.4
FW2 (Cafeteria2)	65.0±1.2	35.0±1.2	94.6±1.2	57.1±1.4	1.6±0.0	9.5±2.2	11.2±0.5	3.8±0.3
FW3 (Kitchen)	49.9±0.8	50.1±0.8	92.4±0.8	32.2±1.2	1.2±0.0	12.7±2.0	14.2±0.6	5.2±0.4

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.

2.2. Pretreatment of FWs with Commercial Enzymes

In order to gauge enzymatic saccharification degree of the mixed food waste (namely FW2 in Table 2), the commercial enzymes, α-amylase and glucoamylase from Genencor, Danisco Singapore Pte Ltd (Singapore) were used to treat FW2 (food waste from cafeteria) sequentially. The optimal pHs for glucoamylase and α-amylase were 5.0-5.8 and 4.2-4.8, respectively. Amylase activity was assayed according to the method by Bernfeld (1955). One unit (1 U) of α-amylase activity was defined as the amount of enzyme releasing 1 μmol glucose equivalent in 1 minute under the assay conditions.

To maximize glucose yield, Response Surface Methodology (RSM) was employed in this study to optimize operation parameters of enzymatic hydrolysis of FW2 with α-amylase and glucoamylase. Experiments were carried out under different operational conditions with four independent variables, i.e. substrate loading, α-amylase loadings, pretreatment temperature and duration. The roles of each variable, their interactions in fermentation were also analyzed with a second-order polynomial model.

Enzymatic pretreatments were conducted in 50 mL Duran bottles placed in a water bath. The suspensions were prepared by mixing the desired amounts of food waste, 100 mM sodium

acetate buffer (pH 5) and α-amylase. Samples were taken at different time intervals. After the liquefaction by α-amylase, glucoamylase treatment was conducted using a fixed enzyme at a dose of 5U/g FW for 2 hours. To optimize the saccharification reaction as well as to maximize the glucose production, the effects of solid and enzyme loadings, temperature and time were investigated through 30 experiments which were based on central composite design. The experimental conditions and the responses are presented in Table 3.

Table 3. Central composite design with observed and predicted responses of glucose yields. Each row corresponds to a single experiment.

					Glucose yield (mg/g FW)	
Run	X_1 [a] Actual (coded)	X_2 [b] Actual (coded)	X_3 [c] Actual (coded)	X_4 [d] Actual (coded)	**Experimental**	**Predicted**
1	10 (+1)	10 (-1)	3 (+1)	60 (-1)	111.5	112.5
2	10 (+1)	50 (+1)	3 (+1)	60 (-1)	127.4	127.6
3	0.5 (-1)	50 (+1)	3 (+1)	60 (-1)	138.2	132.7
4	0.5 (-1)	10 (-1)	3 (+1)	60 (-1)	19.1	23.2
5	10 (+1)	10 (-1)	0.5 (-1)	60 (-1)	59.7	60.6
6	10 (+1)	50 (+1)	0.5 (-1)	60 (-1)	128.8	135.4
7	0.5 (-1)	10 (-1)	0.5 (-1)	60 (-1)	1.8	0.6
8	0.5 (-1)	50 (+1)	0.5 (-1)	60 (-1)	110.5	108.3
9	0.5 (-1)	50 (+1)	0.5 (-1)	95 (+1)	81.0	78.0
10	0.5 (-1)	10 (-1)	0.5 (-1)	95 (+1)	131.4	135.3
11	10 (+1)	10 (-1)	3 (+1)	95 (+1)	189.4	195.3
12	10 (+1)	50 (+1)	0.5 (-1)	95 (+1)	113.0	114.8
13	0.5 (-1)	10 (-1)	3 (+1)	95 (+1)	133.9	139.0
14	10 (+1)	50 (+1)	3 (+1)	95 (+1)	120.7	116.8
15	0.5 (-1)	50 (+1)	3 (+1)	95 (+1)	84.8	87.3
16	10 (1)	10 (-1)	0.5 (-1)	95 (+1)	141.9	137.4
17	14.75 (+α)	30 (0)	1.75 (0)	77.5 (0)	136.8	135.3
18	0.0 (-α)	30 (0)	1.75 (0)	77.5 (0)	132.7	134.6
19	5.25 (0)	70 (+α)	1.75 (0)	77.5 (0)	94.8	95.3
20	5.25 (0)	5 (-α)	1.75 (0)	77.5 (0)	31.0	30.8
21	5.25 (0)	30 (0)	4.25 (+α)	77.5 (0)	139.1	136.3
22	5.25 (0)	30 (0)	0 (-α)	77.5 (0)	79.2	79.9
23	5.25 (0)	30 (0)	1.75 (0)	112.5 (+α)	80.9	84.1
24	5.25 (0)	30 (0)	1.75 (0)	42.5 (-α)	17.0	18.9
25	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	138.6	123.5
26	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	132.8	123.5
27	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	113.0	123.5
28	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	131.7	123.5
29	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	106.8	123.5
30	5.25 (0)	30 (0)	1.75 (0)	77.5 (0)	125.6	123.5

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

A reduced cubic model was used to fit the results. A regression equation (Eq. 1) was generated through the analysis of variance (ANOVA), showing the relationship of the glucose yield to substrate & enzyme loadings, time and temperature.

$$Y = +126.01 + 77.28 * X_1 + 40.49 * X_2 + 23.22 * X_3 + 15.63 * X_4 - 10.14 * X_{12} + 4.05 * X_{13} - 0.082 * X_{14} - 3.41 * X_{23} - 31.45 * X_{24} - 3.11 * X_{34} - 32.08 * X_{11} X_2 - 24.12 * X_1 X_{22} \quad (1)$$

where X_1, X_2, X_3 and X_4 are independent variables representing the α-amylase loadings, substrate loadings, time and temperature, respectively, and Y is the glucose yield (mg/g FW).. The ANOVA results showed that this model can adequately predict the response variables in terms of coded factors with 95% confidence at the level of $P < 0.001$ (Table 4). The model's F-value of 14.99 implies that the model is significant, and there is only a 0.01% chance of that a "model F-value" at this large could occur due to noise. Values of "Prob>F" less than 0.05 indicate that the model terms are significant, while values greater than 0.1 indicate that the model terms are not significant. As such, Table 4 shows 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 all the significant terms. The coefficient of determination (R^2) for the enzyme activity was calculated as 0.98, showing that the proposed model can explain 98% of variability in the response. Fig. 1 is a parity plot showing the correlation between the experimental and predicted values of the response, so that the points cluster around the sloping line, exhibiting the good fit of the model. Similarly, Fig. 11 also 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 is able to represent 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 signal-to-noise ratio of 16.62 was obtained, clearly indicating an adequate signal. Therefore, the model generated (Eq. 1) can be applied to guide experimental design.

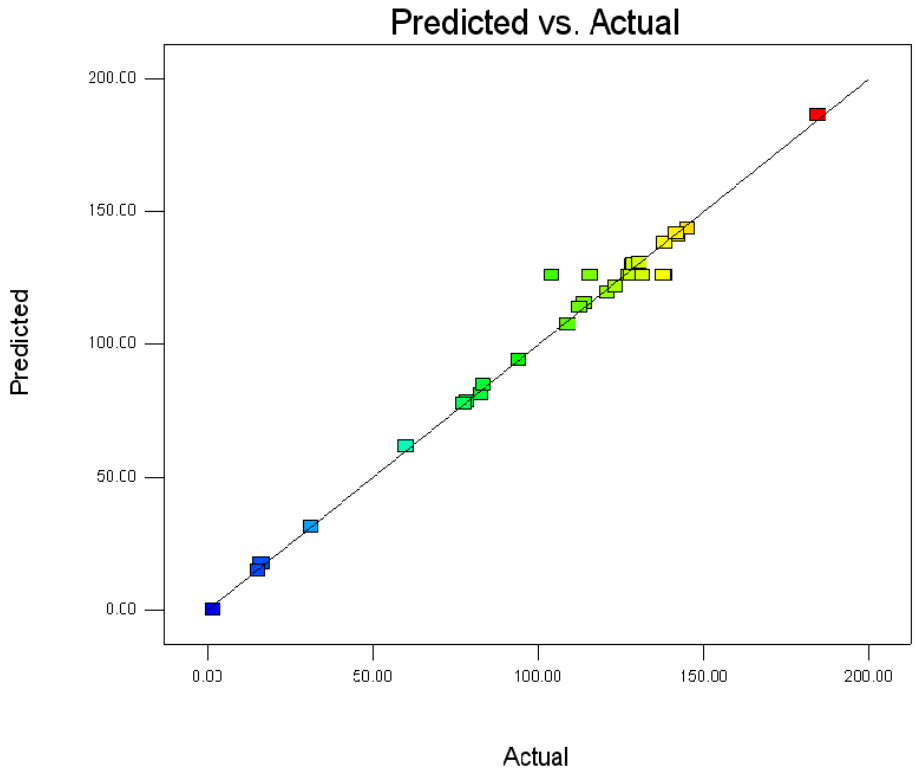


Figure 1. The observed versus the predicted glucose yields under the experimental conditions.

It appears from Table 4 that all the independent variables have a significant effect on the response (e.g. glucose yield in Eq. 1). The positive coefficients for X_1, X_2, X_{13} and X_{24} indicate a positive effect on the saccharification. However, the negative coefficients for X_{12}, X_{14}, X_{23}, X_{34} , X_{112} and X_{122} suggest a negative effect on the response concentration.

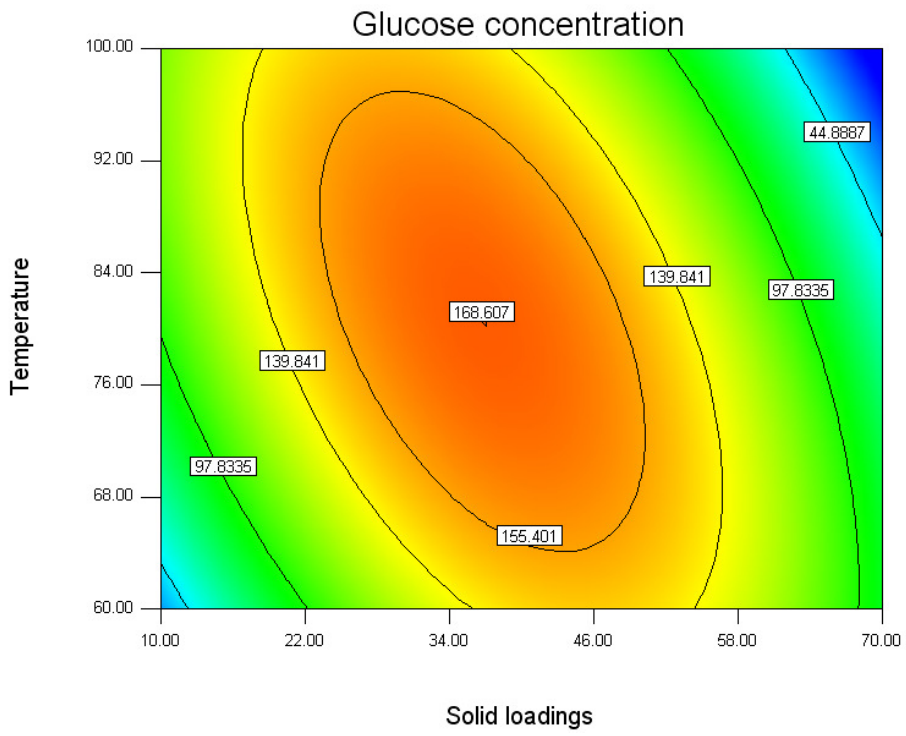
The response surface plots (Fig. 2) are helpful for better understanding the main and interaction effects of two variables. As can be seen in Figure 1A, a glucose yield of 168.6 mg/g FW was obtained at an enzyme loading of 7.25 U/g FW within 105 min. At 80^{o}C, increased solid loadings from 20 to 35% resulted in 18% increase in the glucose yield (Figure 2A). However, when the substrate loadings were increased from 35 to 50%, 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 prevents α-amylase action on FW. The glucose yield was increased by 14.4% (139.8 to 160 mg/g FW) with increasing the residence time from 30 to 90 min, while a further 7.5% (160 to 172 mg/g FW) increase was observed when the reaction time was prolonged from 90 to 150 min (Figure 2B) at a dose of 7.25 U/g FW α-amylase and 80°C. At the solid

loading less than 25%, the glucose yield could not be further improved with prolonged reaction time, and peaked at 168 mg/g FW. Similar trend was also observed at the solid loading higher than 45%. These in turn suggest that the optimal solid loadings should be in between 25-45% for efficient hydrolysis of FW. It was found in Figs. 2C and 2D that the applied α-amylase loadings were related to reaction time and temperature, and the optimal α-amylase loadings should be in the range of 7.3 and 13.2 U/g FW. At the 35% solid loading, the highest glucose yield of 203 mg/g FW was obtained with 10.66 U/g FW enzyme loadings at 83.8^{o}C within 105 min. Further increase in the glucose yield was negligible at the reaction time longer than 3 hours (Figure 2D). At the optimum temperature of (83.8^{o}C), the highest glucose yield of 217 mg/g FW can be expected using 22.41% solid loadings for 90 min (Fig. 2E).

Table 4. ANOVA for glucose yield during enzymatic hydrolysis of FW as a function of α-amylase loadings (X_1), solid loadings (X_2), time (X_3) and temperature (X_4).

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 loadings	1646.22	1	1646.22	10.50	0.0177*
X_2- solid loadings	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			

*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.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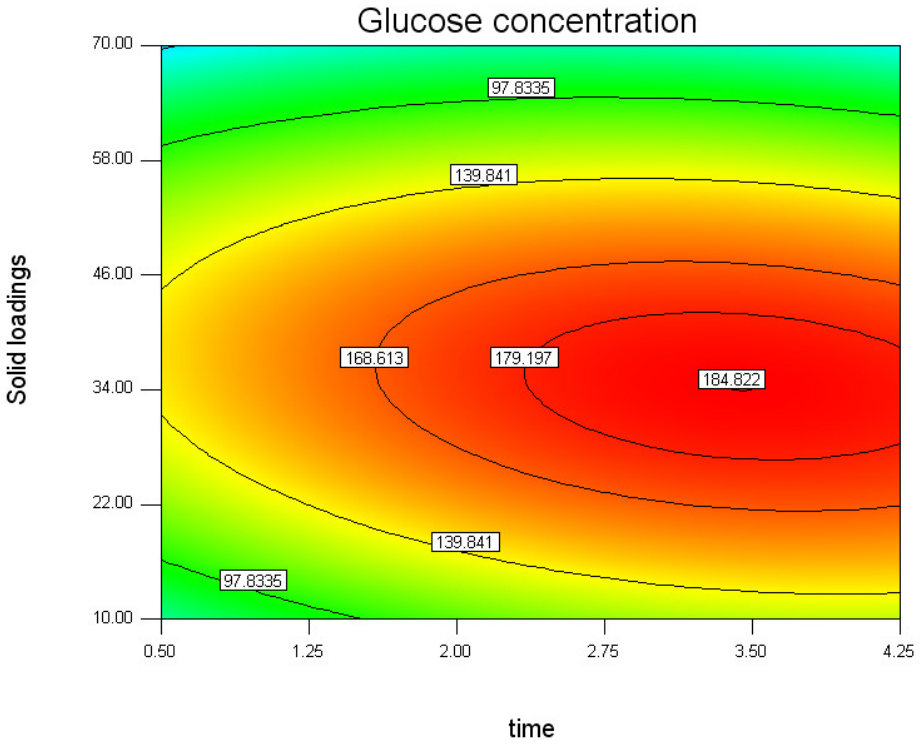
A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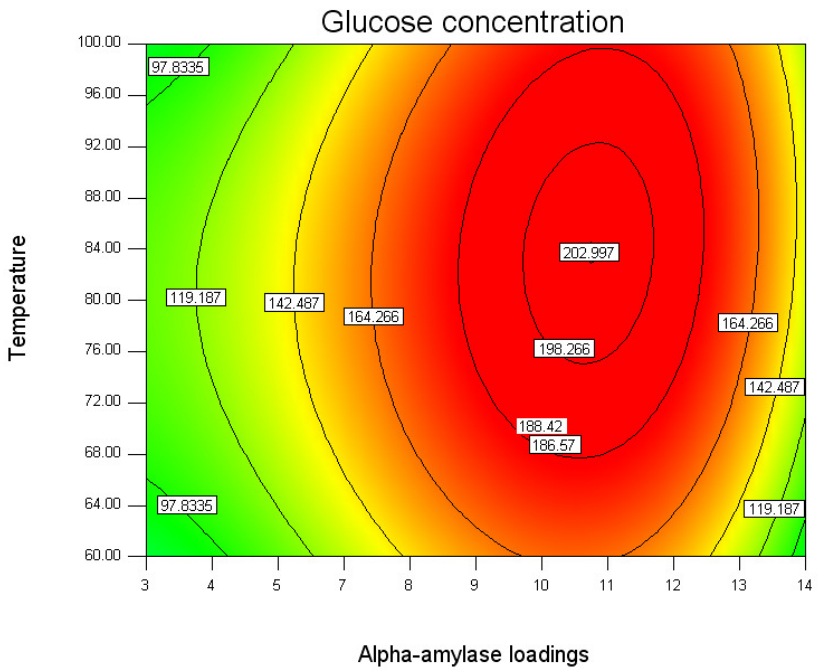
B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60.00
3
4
5
7
8
9
10
11
12
13
14
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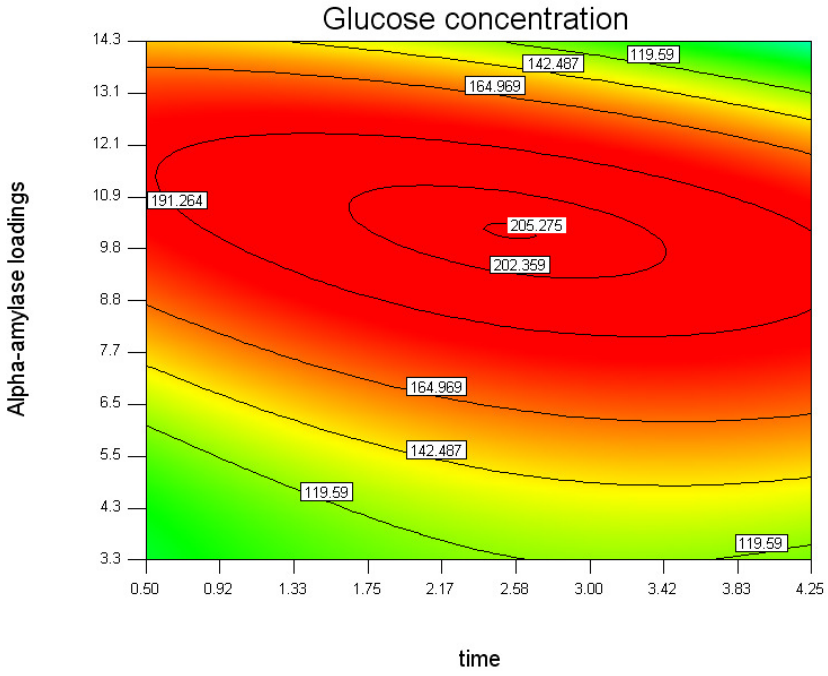
C


Glucose concentration
Alpha-amylase loadings
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3.3
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1.75
2.17
2.58
3.00
3.42
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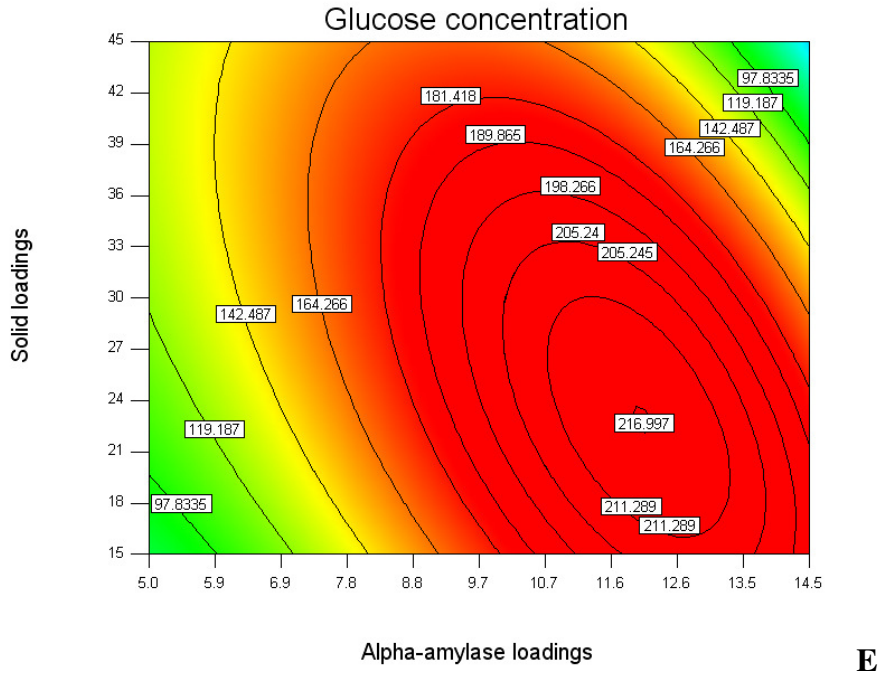
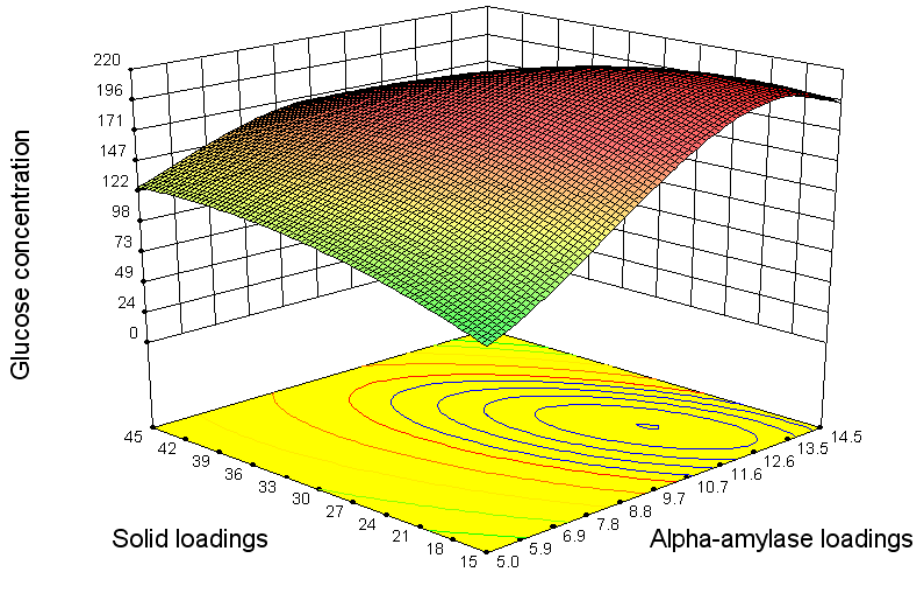
119.59

D



E



F

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^{o}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 83.79^{o}C (2D), and solid loadings and α-amylase loadings at 83.79^{o}C for 1.5h (2E & 2F) on glucose yield from domestic FW.

In summary, enzymatic hydrolysis of domestic FW with α-amylase and glucoamylase was successfully modeled using central composite design and response surface methodology. The optimum conditions predicted by the model were then validated experimentally. Under the identified optimal (83.8^{o}C, 22.4% solid loadings, 12.55 U/g FW α-amylase loadings and 90 min reaction), a maximum glucose yield of 217 mg/g FW was obtained, which shows a yield increase of 43% as compared 123.48 mg/g FW on average achieved in the non-optimized controls. Besides, 98% of the total starch in FW could be saccharified

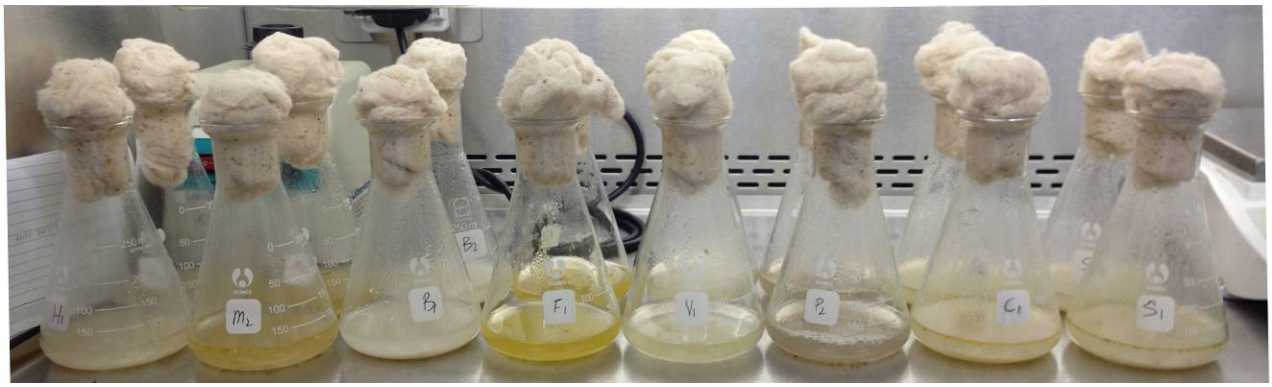
2.3. In-house Glucoamylase Production

In order to determine the best substrate and fermentation mode for glucoamylase production, both submerged and solid-state fermentations were conducted using the FWs described above. The enzyme production studies were conducted with *Aspergillus awamori*, which is one of the well-known glucoamylase producing microorganisms.

2.3.1. Submerged Fermentation

Submerged fermentations of food wastes were conducted using sterilized medium in 250 mL flasks containing 5% FW (w/v) in 50 mL, 100 mM sodium acetate buffer (pH 5.0) (Figure 3). To start, 1 mL fungal inoculum was added to each flask and incubated at 30^{o}C and 300 rpm for 6 days. The samples were taken aseptically and stored at -20^{o}C until determination of the enzyme activity.

Mycelium formation was observed even on second day of the fermentation in each experiment set, showing that the fungus can grow in the FW. The glucoamylase activity profiles are given in Figure 4. In general, the highest enzyme activities were obtained with cake and bread as substrates. On the first day, significant enzyme production was determined on bread and fruit containing media, while lower activities were observed on the others. On the second and third days, enzyme activities on potato containing media started to increase. The highest activities were obtained on 4th day of the fermentation, e.g. 43.2 U/g FW on cake, 42.8 U/g FW on bread, 17.3 U/g FW on fruit 23.6 U/g FW on kitchen FW. The enzyme production from FWs having high lipid content, such as savory and FW from cafeteria, was not significantly low.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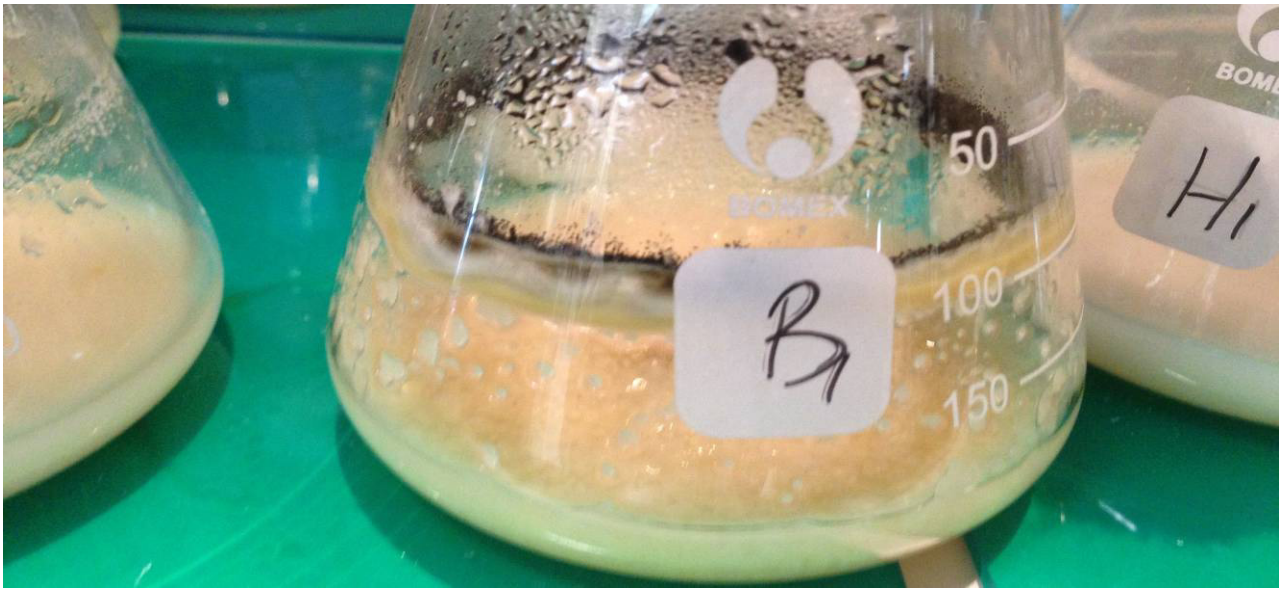

Figure 3. Experiment sets for submerged enzyme production studies (day 0, 2, 4 and 6).

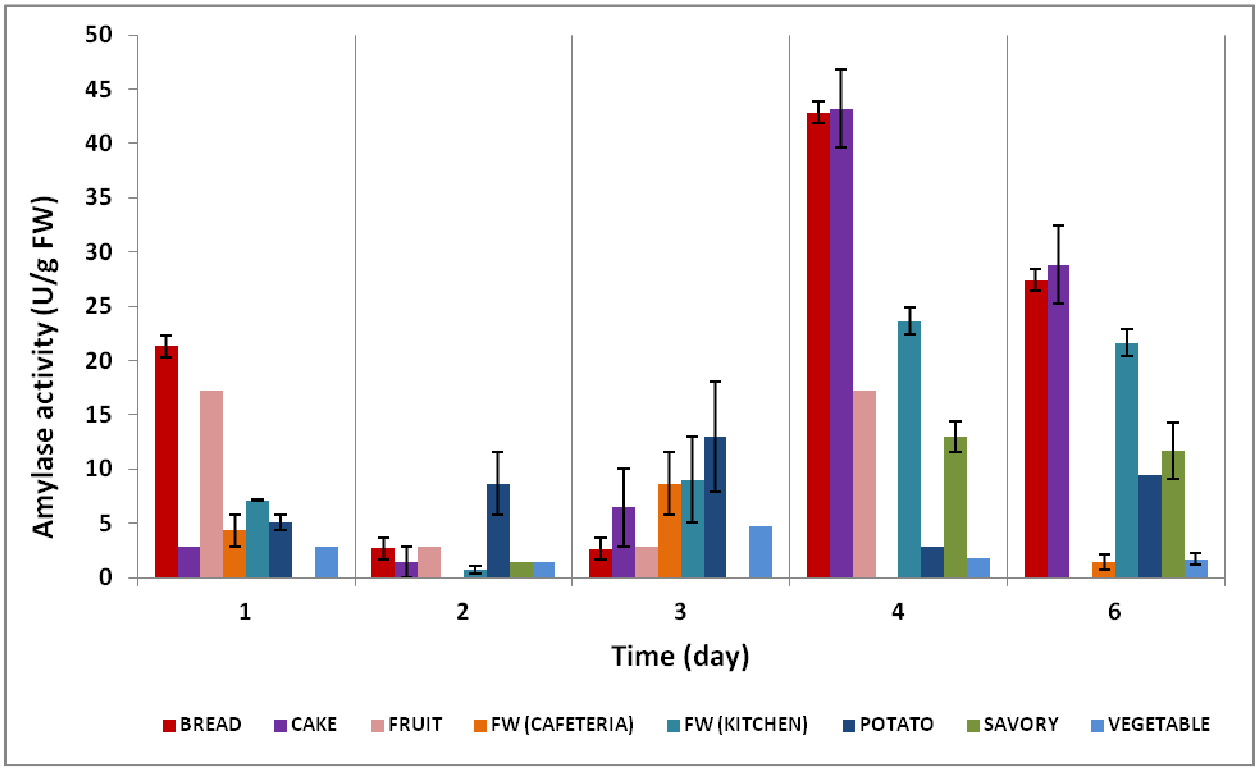

Figure 4. The effect of FWs on GA production in submerged fermentation.

2.3.2. Solid State Fermentation of FW

Solid-state fermentations of food wastes were conducted after sterilizing the fermentation media (i.e. FW with 70% TS) in capped bottles. 1 mL of fungal inoculum was then added to the sterile

medium and mixed with a sterile spatula aseptically. 5g FW was transferred to each petri plate aseptically and the petri dishes were incubated at 30^{o}C for 10 days. Mycelium formation was observed even on first day of the fermentation in each experiment set, showing that the fungus can grow in each FW studied (Figure 5). After having such positive results, systematic experiments will be carried out in Petri dishes to find the optimum conditions for enzyme production. The spore formation started after the second day of the fermentation and spores covered the whole substrate after the 6th day of the fermentation.

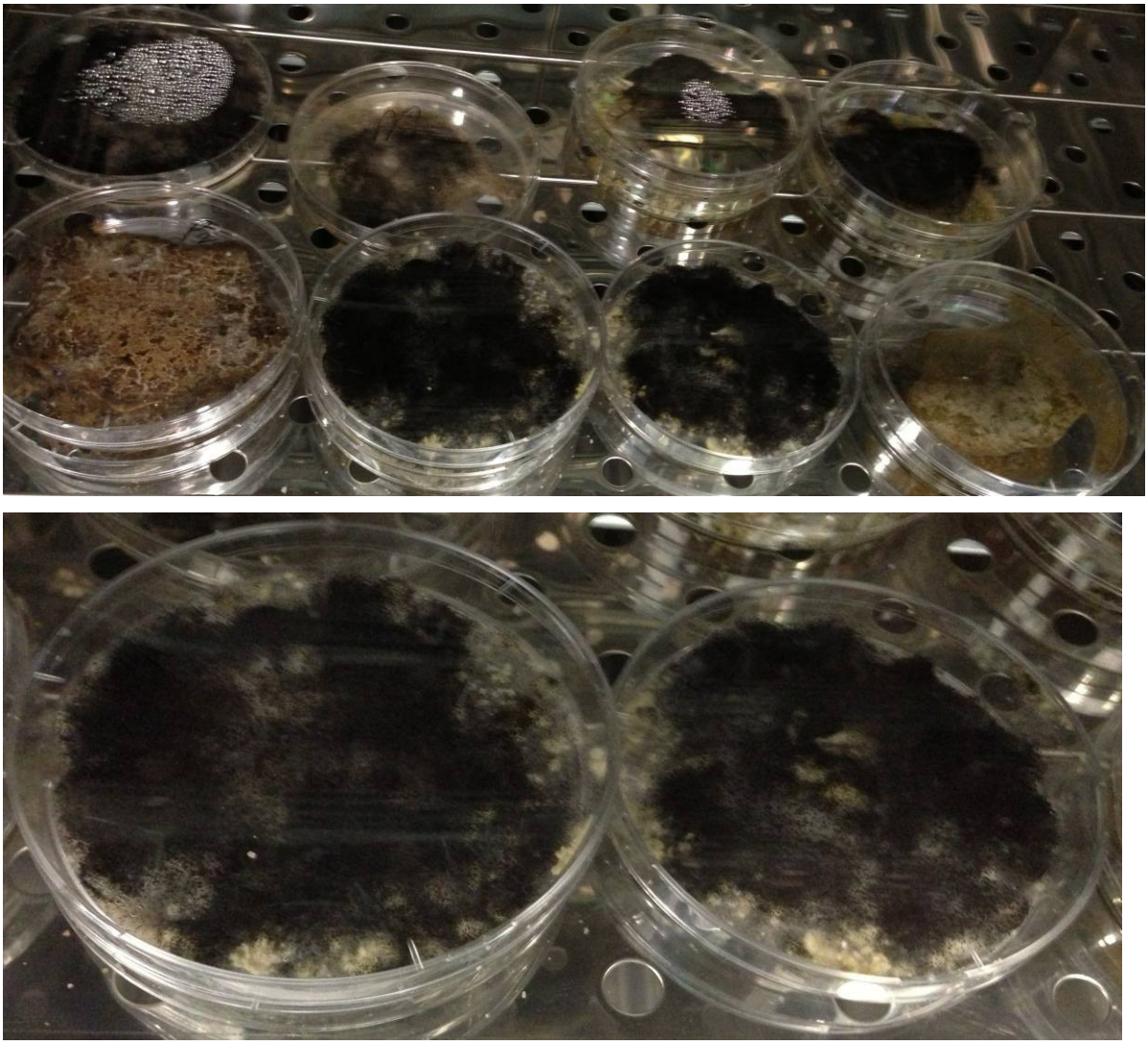

Figure 5. Experiment sets for solid state enzyme production studies (day 0, 2, 4 and 8).

Figure 6 shows the glucoamylase activity profiles. In general, the highest enzyme activities were obtained with cake, mixed food waste (cafeteria) and fruit. For example, the highest enzyme activity of 155.8 U/g FW was obtained in the solid fermentation of cake as sole substrate, while only 43.2 U/g FW was achieved in submerged fermentation of the same FW. This suggests that solid-state fermentation is a better option than submerged one for enzyme production. Thereby, the parameters will be optimized to improve the solid-state enzyme production in subsequent studies.

Although the highest glucoamylase activity was observed in the solid-state fermentation of cake, no activity on cake containing media was observed on the first day of the fermentation, while

significant activities were found on bread (90.9 U/g FW) and fruit (85.6 U/g FW) containing media. Cake contains significant amount of soluble sugar, thus the fungi might consume it first without production of glucoamylase on day 1. On second day, the enzyme activities on cake containing media increased markedly and reached to 128.4 U/g FW, followed by that in fruit (78.9 U/g FW) and potato (40.8 U/g FW). The enzyme activity reached its peak of 155.8 U/g FW on cake containing media on day 6.

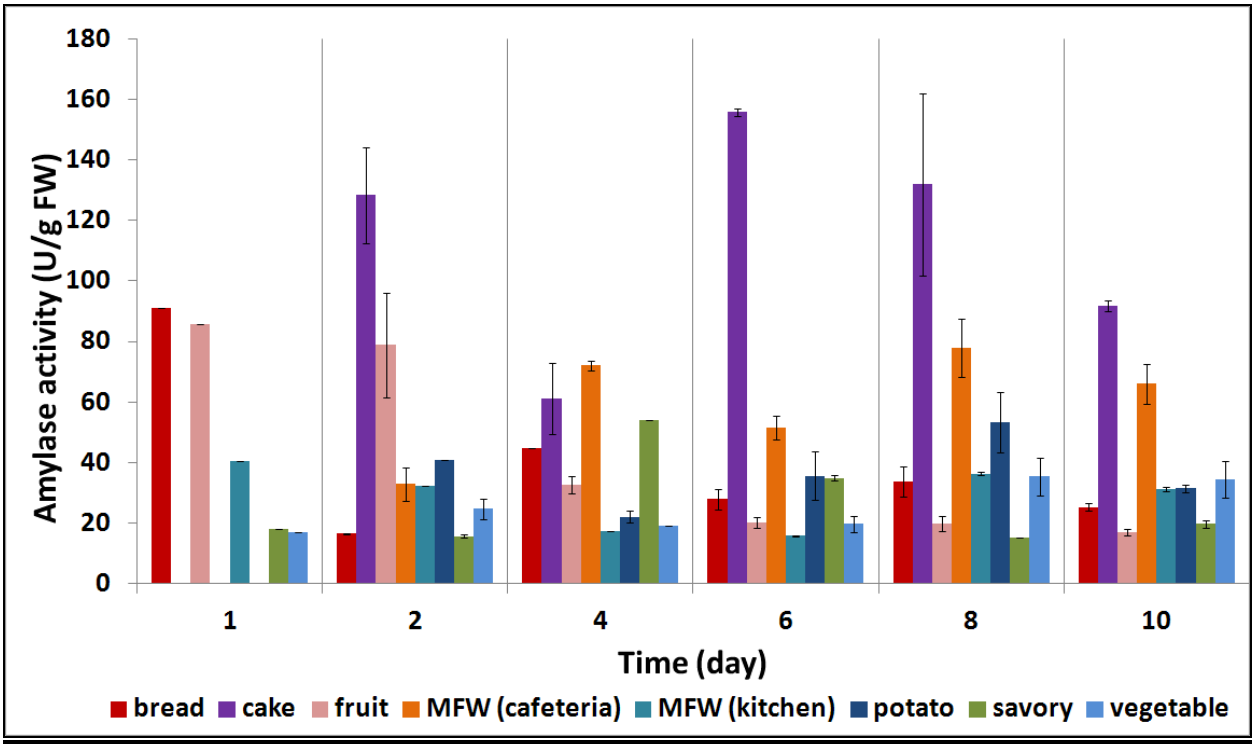

Figure 6. The effect of FWs on GA production in solid state fermentation.

3. Plans for the Next 6 and 12 Months Period

Next 6 Months:

- We are targeting to optimize in-house solid-state enzyme production from cake wastes using *Aspergillus awamori.* For this, RSM design will be used to ease the optimization procedure and to determine the interaction between the parameters.
- The recovery of enzyme cocktails will be investigated. For this, we are planning to use simple autolysis method by heat. Then, the effects of enzyme addition as a solid cake and crude extract will be investigated.

- The produced enzyme cocktails will be used to saccharify mixed type food wastes (from cafeteria and kitchen). For this, the enzyme loading, solid loading, temperature, pH and hydrolysis time will be optimized in two hydrolytic bioreactors.

Next 12 Months:

- Besides *Aspergillus awamori*, microbial strains producing other saccharification enzymes such as α-amylase, cellulase, β-glucosidase and pullulanase, will be cultured and/or co-cultured with *Aspergillus awamori* to produce an enzyme cocktail in order to improve the saccharification yield further.
- The effect of different pretreatments, such as heat, alkaline, microwave, ozonation and ultrasonication on food waste, will be investigated to improve enzyme production and saccharification.
- The produced enzyme cocktails will be used to saccharify mixed type food wastes. For this, the enzyme loading, solid loading, temperature, pH and hydrolysis time will be optimized in hydrolytic bioreactors.
- The residues will be used to produce biogas using anaerobic digestion.
- The final report, publications and project proposal for the continuation of the project will be prepared.

The proposed milestones and deliverables are summarized in Table 5.

Table 5. Updated Gantt chart with completed tasks

Milestones and Deliverables	Year 1				Year 2			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
✓ Literature Review								
✓ Characterisation of KW								
✓ Construction of 2 HRs (2-5L)								
✓ Liquid state fermentation of food waste by *Aspergillus*: characterization of enzymes								
✓ Milestone 4: Solid State fermentation								

of food waste by *Aspergillus* characterization of enzymes								
Optimization of in-house enzymes production								
Enzymatic hydrolysis of KW using in-house enzymes cocktail produced by *Aspergillus*								
✓ Enzymatic hydrolysis of KW using commercially available enzymes for benchmarking								
Deliverable 1: Bench-scale process for sugar production								

4. PERFORMANCE INDICATORS

Items	**Target**	**Achieved**
Numbers of patents or intellectual properties	1	0
Numbers of researchers*	1	1
Numbers of research man-months*	24	24
Numbers of publications in leading journals	2-3	1

*Include researchers, scientists and engineers (RSEs) and research scholars (Masters & PhDs)

In the project proposal it was proposed to produce 1 IP and 2 to 3 article during the project. We have already prepared two articles:

-One comprehensive review article on food waste valorization using biological treatment strategies.

-One article on the optimization of enzymatic glucose production from cafeteria food waste using response surface methodology.

Declaration

I declare that the information of the Development Project as described in the above report is true and to the best of my knowledge.

Associate Prof Liu Yu		
Principle investigator	Signature Principle Investigator	Date

Technology Transfer office (or equivalent)	Signature Technology Transfer office (or equivalent)	Date

Name Research Director	Signature Research Director	Date Research Director

Organization Stamp: _____

<Organization's name>

** Please note that the completeness of the report submitted will help to ensure the efficient processing of the disbursement claim.*

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