ETRP PROGRESS REPORT

Address to:

Director, Environment Technology Office

National Environment Agency

40 Scotts Road, #11-00, Environment Building

Singapore 228231

Attn: ETRP Secretariat

National Environment Agency

Environment Technology Office

40 Scotts Road, #11-00, Environment Building

Singapore 228231

Attn: Project Officer, Ms Teresa Cheong

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: A/P Yu Liu**

Project Period (Please tick the appropriate box)

X December Progress Report for the period *<06/2013>* to *<12/2013>*

□ 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 December 2013, we have completed the optimization of in-situ glucoamylase production from cake waste. Meanwhile, the saccharification level of mixed type food wastes (from cafeteria) was investigated using in-situ produced enzyme solution, and the optimization studies were started. The two bioreactors with essential control software now are ready for the next phase of experiments. The waste volume reduction after enzymatic saccharification was also evaluated.

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. Optimization of in-situ enzyme production

The influence of different food waste such as bread, cake, savory, vegetable, fruit, potato and mixed type food waste (MFW) from a cafeteria on glucoamylase production by *Aspergillus awamori* was tested previously (Figure 1). The incubation time is governed by characteristics of the culture and is based on growth rate and enzyme production. Maximum glucoamylase production normally occurs after 2-5 days of incubation as reported by other researchers working with solid state cultures involving bacteria and fungi (Melikoglu et al., 2013b; Soni et al., 2003). The maximum activity of glucoamylase was obtained using waste cakes on the 4^{th} day of fermentation. Due to a more balanced composition of cake waste with high reducing sugar and protein content, a better fungal growth and higher glucoamylase activity was obtained. As the highest glucoamylase activity was obtained using cake wastes (85.1±6.8 U/gds), following experiment sets were conducted using it.

2.1.1. Effects of particle size

The utilization of the substrate during solid state fermentations by the fungi is not only influenced by its nutritional quality but also by the particle size of the solid substrate (Schmidt and Furlong, 2012). Experimental studies shown in Figure 1 validated that particle size has a direct effect on glucoamylase production during solid state fermentation. The highest glucoamylase activity was measured with a particle size of $0.6 \leq X \leq 1.18$ mm. In solid state fermentations, smaller particle size provides larger contact area. However, reduction in particle size increases the packing density, which causes a reduction in the void space between the particles, which results in reduction in microbial growth and enzyme production (Ruiz et al., 2012). Therefore, there must be an optimum for particle size. As the highest glucoamylase activity was obtained using $0.6 \leq X \leq 1.18$ particle size, it was adjusted to that particle range in the following set of experiments.

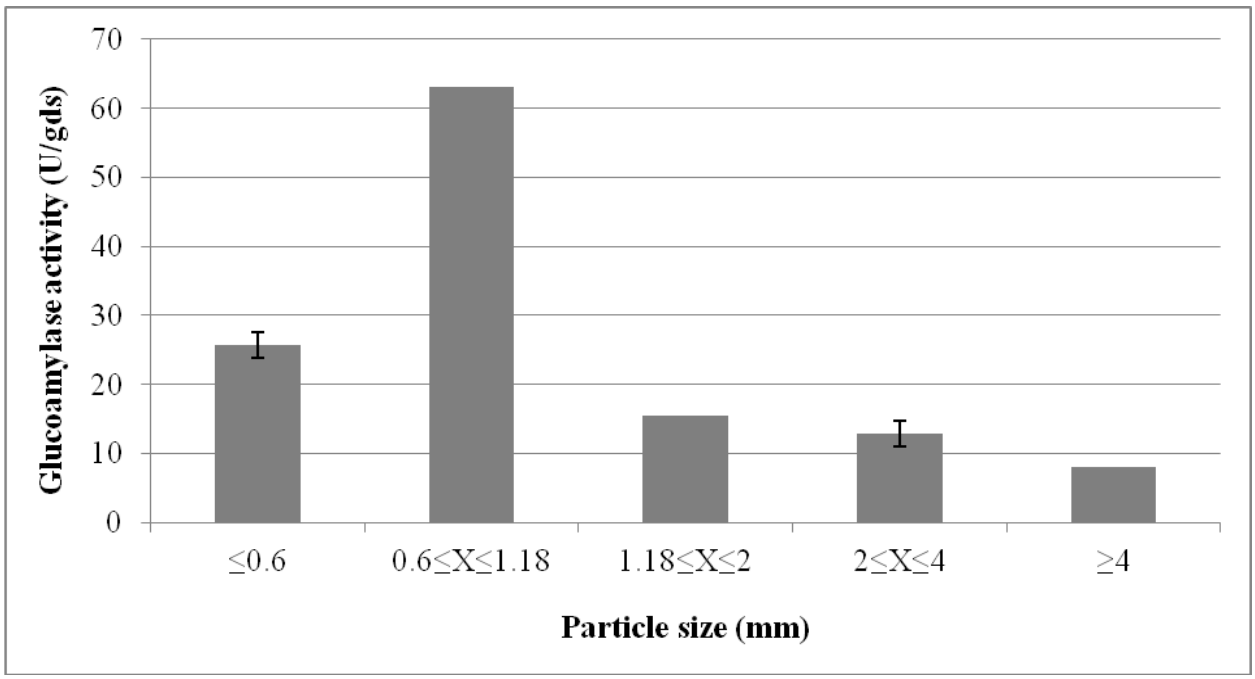


Figure 1. The effect of cake particle size on glucoamylase production using moisture content of 70% (wb), inoculum loading of 10^6/g substrate at neutral initial pH and 30^{o}C for 4 days.

2.1.2. Optimization of glucoamylase production by response surface methodology

To determine the optimum pH, moisture content, inoculum loading and time that maximize glucoamylase activity, thirty experiments were designed using Central Composite Design (CCD). The experimental conditions and the responses are presented in Table 1. A quadratic model was chosen from several models and fitted to the results. The regression equation obtained after the analysis of variance (ANOVA) represented the level of enzyme activity as a function of initial pH, moisture content, inoculum loading and time.

Table 1. Central composite design with observed and predicted responses of glucoamylase activities. Each row corresponds to a single experiment.

Run	X_1 [a] Actual (coded)	X_2 [b] Actual (coded)	X_3 [c] Actual (coded)	X_4 [d] Actual (coded)	Experimental	Predicted
1	6 (-1)	60 (-1)	100000 (-1)	4 (-)	13.73	14.56
2	6 (-1)	60 (-1)	100000 (-1)	6 (+)	10.34	18.73
3	6 (-1)	60 (-1)	1000000 (+1)	4 (-)	4.2	-4.59
4	6 (-1)	60 (-1)	1000000 (+1)	6 (+)	2.06	12.71
5	8 (+1)	60 (-1)	100000 (-1)	4 (-)	36.18	35.15
6	8 (+1)	60 (-1)	100000 (-1)	6 (+)	92.57	84.06
7	8 (+1)	60 (-1)	1000000 (+1)	4 (-)	4.14	3.73
8	8 (+1)	60 (-1)	1000000 (+1)	6 (+)	67.34	65.81
9	6 (-1)	80 (+1)	100000 (-1)	4 (-)	5.76	5.66
10	6 (-1)	80 (+1)	100000 (-1)	6 (+)	5.26	7.42
11	6 (-1)	80 (+1)	1000000 (+1)	4 (-)	7.34	17.60
12	6 (-1)	80 (+1)	1000000 (+1)	6 (+)	33.14	32.53
13	8 (+1)	80 (+1)	100000 (-1)	4 (-)	26.62	17.72
14	8 (+1)	80 (+1)	100000 (-1)	6 (+)	57.08	64.24
15	8 (+1)	80 (+1)	1000000 (+1)	4 (-)	27.46	17.43
16	8 (+1)	80 (+1)	1000000 (+1)	6 (+)	76.24	77.13
17	7 (0)	50 (-α)	550000 (0)	5 (0)	39.9	40.14
18	9 (+α)	70 (0)	550000 (0)	5 (0)	51.00	62.24
19	7 (0)	70 (0)	1000 (-α)	5 (0)	53.41	53.08
20	7 (0)	70 (0)	1100000 (+α)	5 (0)	6.58	15.70
21	7 (0)	70 (0)	550000 (0)	3 (-α)	88.78	79.54
22	7 (0)	70 (0)	550000 (0)	7 (+α)	93.20	88.44
23	5 (-α)	70 (0)	550000 (0)	5 (0)	8.44	-2.92
24	7 (0)	90 (+α)	550000 (0)	5 (0)	42.88	42.52
25	7 (0)	70 (0)	550000 (0)	5 (0)	93.67	88.44
26	7 (0)	70 (0)	550000 (0)	5 (0)	74.86	88.44
27	7 (0)	70 (0)	550000 (0)	5 (0)	93.67	88.44
28	7 (0)	70 (0)	550000 (0)	5 (0)	81.38	88.44
29	7 (0)	70 (0)	550000 (0)	5 (0)	93.67	88.44
30	7 (0)	70 (0)	550000 (0)	5 (0)	117.86	88.44

[a] Coded values of initial pH, [b] moisture content (%, w/w), [c] inoculum loading (inoculum/g substrate), [d] time (day).

On the basis of their P-value, R^2, SD and predicted sum of square values, the adequacy of the quadratic regression model was found to be significant for glucoamylase production. The statistical significance of the ratio of mean square variation due to regression and mean square residual error was tested using the ANOVA. The associated P-value is used to estimate whether

F is large enough to indicate statistical significance. If P-value is lower than 0.05, it indicates that the model is statistically significant. The ANOVA result for the glucoamylase production system shows the model F-value of 21.96 indicating that the model is significant (Table 2). There is only a 0.01% chance that a "Model F-Value'' this large could occur due to noise. The P-values less than 0.05 indicated that model terms (X_1, X_4, X_{14}, X_{23}, X_{11}, X_{22}, X_{22}, X_{33} and X_{44}) are significant, and the larger P-values (>0.05) of the regressions X_2, X_3, X_{12}, X_{13}, X_{24} and X_{34} suggested their insignificance in the model. The coefficient of determination (R^2) for the enzyme activity was calculated as 0.9565, showing that the fitted model could explain 95.65% of variability in the response. Moreover, the high value of R^2 indicates that the quadratic equation is able to represent the system under the given experimental domain. An adequate precision of 12.74 for the enzyme activity was recorded. A value greater than 4 is desirable in support of the fitness of the model (Muthukumar et al., 2003). The adjusted R^2 corrects the R^2 value for the sample size and the number of terms used in the selected model. If there are many terms in the model and the sample size is not large enough, the adjusted R^2 may be clearly smaller than R^2. The P-value was used to determine the significance of related coefficients. If the P-value is lower than 0.05, the model and the corresponding coefficient is statistically significant (Khuri & Cornell, 1987). The Coefficient of Variation (CV) indicates the degree of precision with which the treatments are compared. Usually, the higher the CV value, the lower is the reliability of experiment. In this experiment, a CV value of 22.81 indicates an adjusted greater reliability of the experiments performed. The table also shows a term for residual error, which measures the amount of variation in the response data left unexplained by the model. The analysis shows that the form of the model chosen to explain the relationship between the factors and the response is correct.

Table 2. ANOVA for glucoamylase production as a function of initial pH (X_1), moisture content (X_2), inoculum loading (X_3) and time (X_4).

Source	Sum of Squares	DF	Mean Square	F Value	p-value Prob > F
Model	31703.19	14	2264.51	21.96	< 0.0001
X_1- pH	6367.44	1	6367.44	61.75	< 0.0001
X_2- moisture content	8.52	1	8.52	0.083	0.7780
X_3- inoculum loading	42.37	1	42.37	0.41	0.5318
X_4- time	6112.04	1	6112.04	59.28	< 0.0001
X_{12}	72.25	1	72.25	0.70	0.4166
X_{13}	149.57	1	149.57	1.45	0.2484
X_{14}	2003.91	1	2003.91	19.43	0.0006
X_{23}	969.39	1	969.39	9.40	0.0084
X_{24}	5.66	1	5.66	0.055	0.8181
X_{34}	173.45	1	173.45	1.68	0.2156
X_{11}	5660.75	1	5660.75	54.90	< 0.0001*
X_{22}	3636.26	1	3636.26	35.27	< 0.0001*
X_{33}	3628.09	1	3628.09	35.19	< 0.0001*
X_{44}	2730.15	1	2730.15	26.48	0.0001
Residual	1443.52	14	103.11		
Lack of Fit	1104.55	9	122.73	1.81	0.2660
Pure Error	338.97	5	67.79		
Corrected total	33146.71	28			

*Significant variable; DF, degree of freedom; determination coefficient (R^2), 0.9565; adjusted determination coefficient (R^2adj), 0.9129; coefficient of variation (CV), 22.81; adequate precision ratio, 12.74.

The equation in terms of actual factors (confidence level above 95%) as determined by Design of expert software is given below:

$$\text{GA Activity (U/gds)} = -1366.16 + 184.69*X_1 + 17.38 * X_2 + 8.02*10^{-6} *X_3 + 39.82*X_4 - 0.21* X_1*X_2 - 6.79*10^{-6}*X_1*X_3 + 11.19*X_1*X_4 + 1.73*10^{-6}* X_2*X_3 - 0.06*X_2*X_4 + 7.32*10^{-6}*X_3*X_4 - 14.7* X_1^2 - 0.12*X_2^2 - 1.11*10^{-10}*X_3^2 - 10.21*X_4^2$$

where X_1, X_2, X_3 and X_4 are independent variables representing the pH, moisture content, inoculum loading and time, respectively. The negative coefficients for X_{12}, X_{13}, X_{24}, X_{11}, X_{22},

X_{33} and X_{44} demonstrate the existence of quadratic and linear interaction effects that decrease the response quantity, while the positive coefficients for X_{14}, X_{23} and X_{34} expose the existence of quadratic interaction effects that enhance the activity of glucoamylase. Figure 2 shows the correlation between the experimental and predicted values of the response. The points close to the line indicate a good fit between the experimental and predicted data.

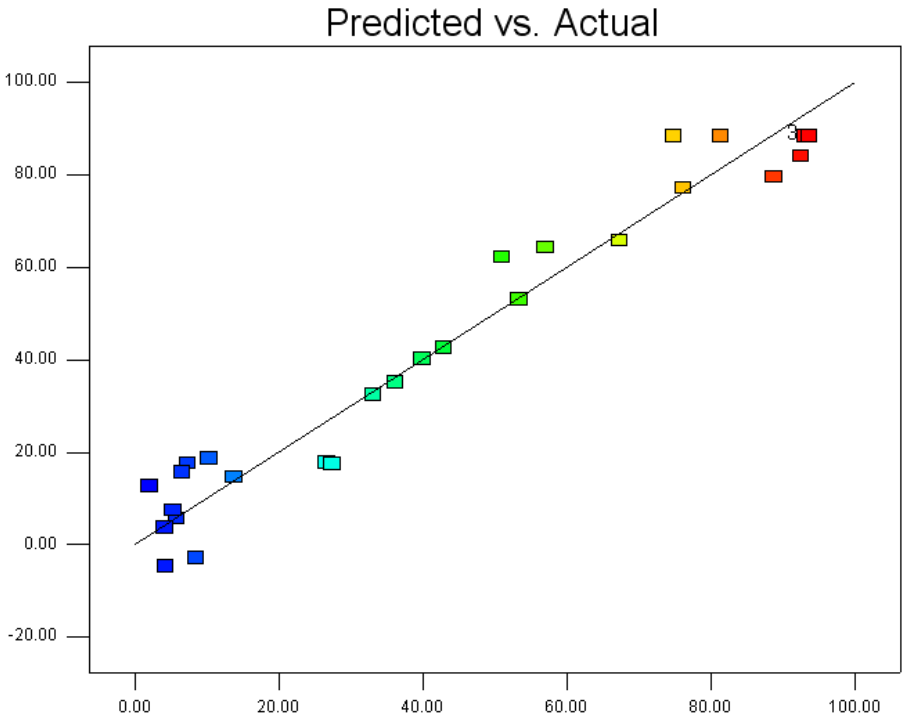
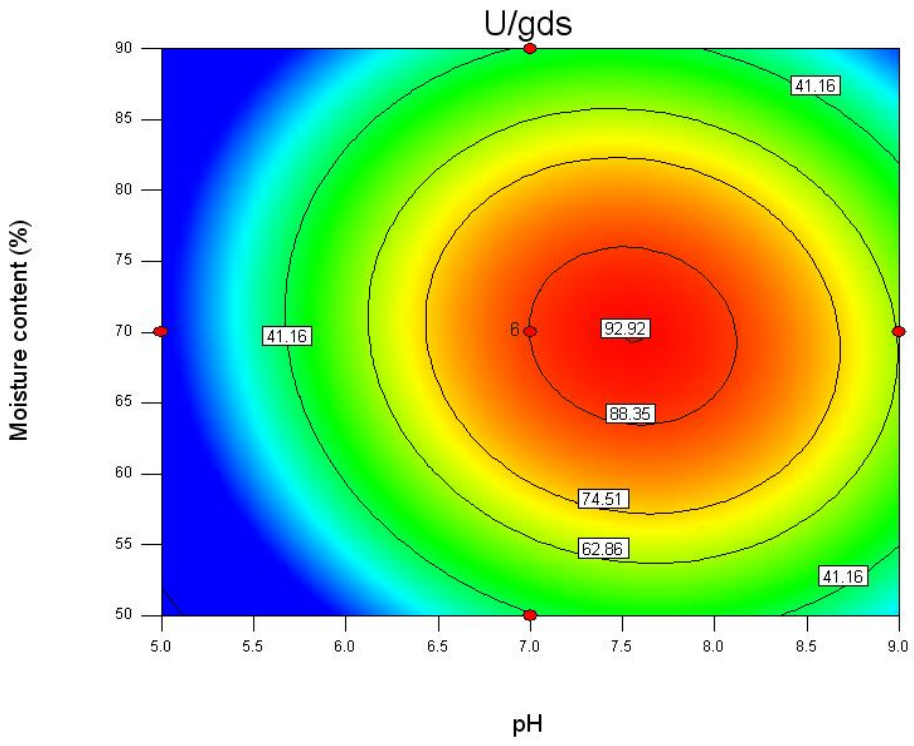


Figure 2. The observed (X axis) versus the predicted (Y axis) glucoamylase activities under the experimental conditions.

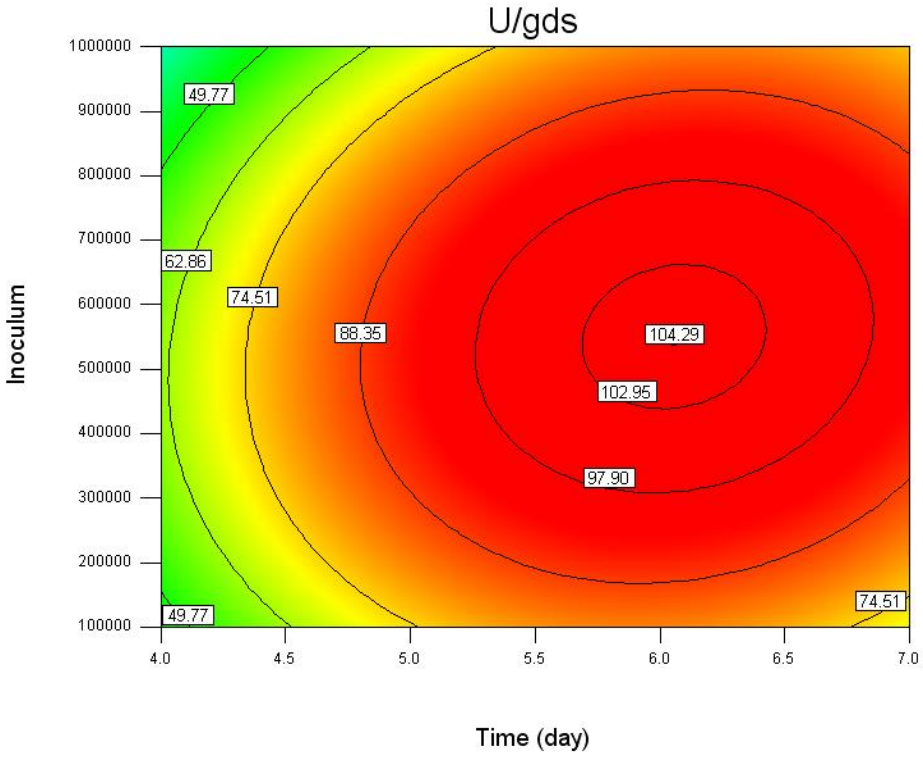
The optima of the variables for which the responses are maximized are represented by the contour plots (Figure 4). The contour plot of the moisture content and pH effect on the activity of glucoamylase illustrates that the neutral pHs led to higher enzyme activity using an initial moisture content of around 66-74% (wb) (Figure 3A). The maximum activity of 92.92 U/gds was determined at pH 7.5 using initial moisture content of 69.6%. Lower initial moisture content provides lower solubility of the nutrients while higher moisture contents cause decreased porosity and decrease in gas exchange. The moisture content range is consistent with the levels reported in the literature for solid state fermentation of waste bread and wheat flour by *A. awamori* (Melikoglu et al., 2013b; Wang et al., 2009). Generally, the initial pH for glucoamylase

production by *A. awamori* using SSF is adjusted to neutral pHs as the fungus grows well at such pHs. Since the maximum activity of 92.92 U/gds was determined at pH 7.5 using initial moisture content of 69.6%, these conditions were kept constant in the subsequent studies to find the optimum inoculum loading and incubation time.

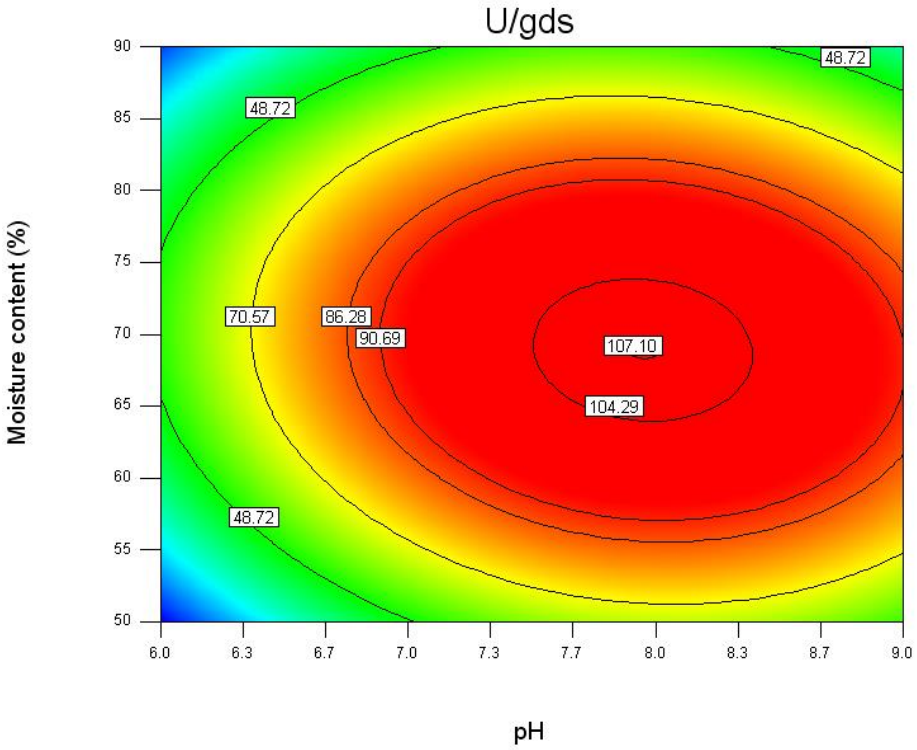
The glucoamylase production increased by using an inoculum loading of $2*10^5$ to $9*10^5$/gs for 5 to 7 days and the maximum glucoamylase activity of 104.29 U/gds was obtained using $5.2*10^5$/gs inoculum on 6^{th} day of the fermentation (Figure 3B). During the fermentation, medium pH, nutrient concentration, temperature, moisture content, and physical structure of the raw material changes constantly. All these parameters affect microbial growth and enzyme production. According to Melikoglu et al. (2013b), the growth of *A. awamori* on bread pieces increased exponentially between the 3^{rd} and 5^{th} days and glucoamylase production reached its maximum level on the 6^{th} day of the fermentation. However, as the medium pH was not controlled, the pH is decreasing during this period. (Melikoglu et al., 2013b) They reported that the pH decreased to 3.8 on the 5^{th} day of the fermentation. This may be one of the major causes of deceleration of the growth and enzyme production after 6^{th} day of the fermentation. Therefore, the effect of initial pH was evaluated using the optimized parameters and it was predicted that the glucoamylase activity increased from 90.69 U/gds to 107.1 U/gds using initial pH of 7.9 instead of pH 7.0 (Figure 3C). The pH reached 4.5 after the 5^{th} day of the fermentation when the initial pH was 8 and 9. On the other hand, the pH decreased to 3.5 and 4 when the initial pH was adjusted to 6 and 7, respectively. This explains why the microbial growth and glucoamylase production was enhanced using an initial pH of 7.9.


U/gds
Moisture content (%)
pH
90
85
80
75
70
65
60
55
50
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
41.16
41.16
92.92
88.35
74.51
62.86
41.16

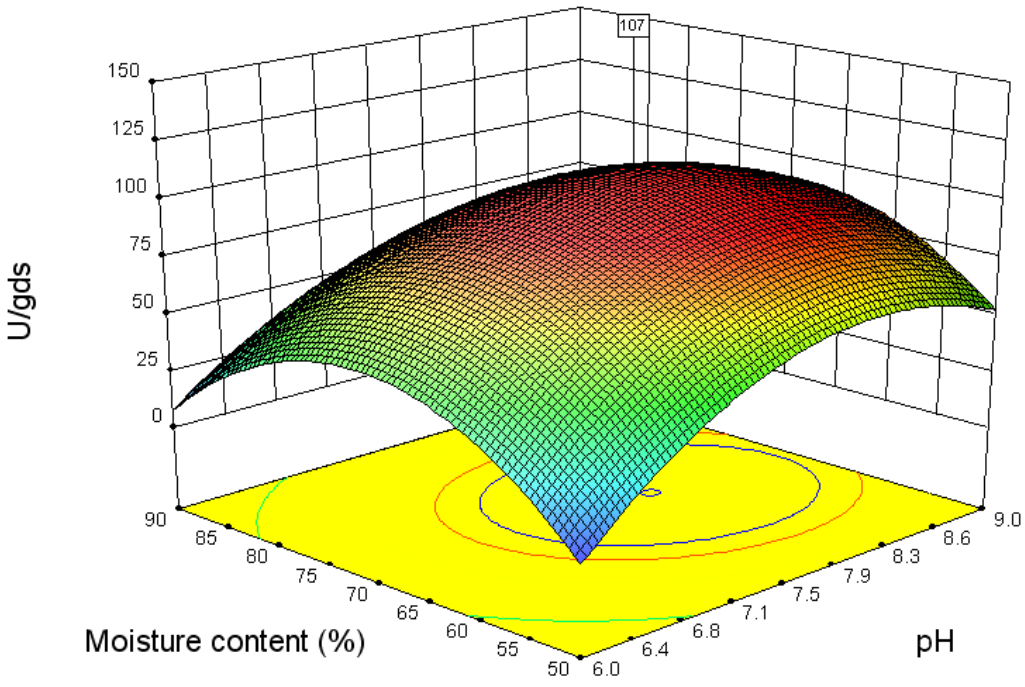
~~4A~~3A


U/gds
Inoculum
Time (day)
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
4.0
4.5
5.0
5.5
6.0
6.5
7.0
49.77
62.86
74.51
88.35
104.29
102.95
97.90
74.51
49.77

~~4B~~3B



4C3C



4D3D

Figure 3. Contour plots, described by Eq. (2), representing the effect of initial pH and moisture content using inoculum loading of $5*10^5$/g substrate for 5 days (4A3A); inoculum loading and incubation time using the initial moisture content of 69.6% and pH of 7.5, (4B3B); inoculum

loading and pH using initial moisture content of 69.6% for 6 days (~~4C~~3C); initial pH and moisture content using inoculum loading of $5.2*10^5$/g substrate for 6 days (~~4D~~3D) on glucoamylase activity from cake waste.

2.1.3. Validation of the response model

To evaluate the accuracy of quadratic polynomial model, a verification experiment was conducted under the predicted optimal conditions and the result was 108.47 U/gds which is 1.37% higher than the predicted value. This is higher than values reported by (Wang et al., 2009) for the same fungus using wheat flour and similar to those reported by (Melikoglu et al., 2013b) on bread pieces. This high degree of accuracy obtained confirms the validity of the model with minor discrepancy due to the slight variation in experimental conditions. The activity obtained is (1.4 fold) higher than the yield obtained by cake wastes at 6^{th} day of the fermentation without optimization suggesting the important role of RSM for rapid screening of important process variables in the optimization studies.

2.2. Saccharification of FWs with In-situ Produced Enzyme

In order to gauge enzymatic saccharification degree of the mixed food waste, in-situ produced enzyme solution (obtained from cake waste) was used. The optimal pH temperature for the saccharification using glucoamylase from *Aspergillus awamori* was reported as 60^{o}C (Melikoglu et al., 2013a). To maximize glucose yield, the effects of different operational conditions such as substrate loading, glucoamylase loading and pretreatment duration were studied.

2.2.1. The effects of substrate loading

Enzymatic pretreatments were conducted in 100 mL Duran bottles placed in a water bath. The suspensions were prepared by mixing the desired amounts of food waste, 100 mM phosphate buffer (pH 7) and the enzyme solution. Glucoamylase treatment was conducted using 10, 20, 30, 40 and 50% (w/v) food waste loadings with 2, 5 and 10U/g FW enzyme loadings for 24 hours. Higher than 50% waste concentrations were not studied as the suspension became very viscous, which certainly would inhibit enzyme's action. The hydrolysis conducted for 24h and samples were taken at different time intervals. The glucose concentration released was increased dramatically with an increase in substrate loading and the hydrolysis continued until 24^{th} hour (Figure 4). The hydrolysis rate was sluggish for the sets using 30, 40 and 50% waste loading

until t=4h and increased dramatically after t=4h. This might be related to elongated starch gelatinization process.

At t=24 hour, maximum glucose concentration of 9.3±0.9, 14.8±0.77, 19.7±0.77 and 39.1±2.93 and 52.3±2.97 g/L was obtained using 10, 20, 30, 40 and 50% (w/v) food waste loadings, respectively. Moreover, the saccharification degree at t=24h was 99.8% for 50% substrate loading. These results demonstrate that there is no substrate inhibition so food waste can be used without much dilution, which in turn decrease the waste water generation. Even at t=12h, the saccharification degree was in the range of 55-88% depending on the waste concentration. Still, the production of maximum glucose concentration is the main target in this study. Thereby, the effects of higher enzyme loadings needed to be evaluated to obtain high glucose concentration in shorter hydrolysis time.

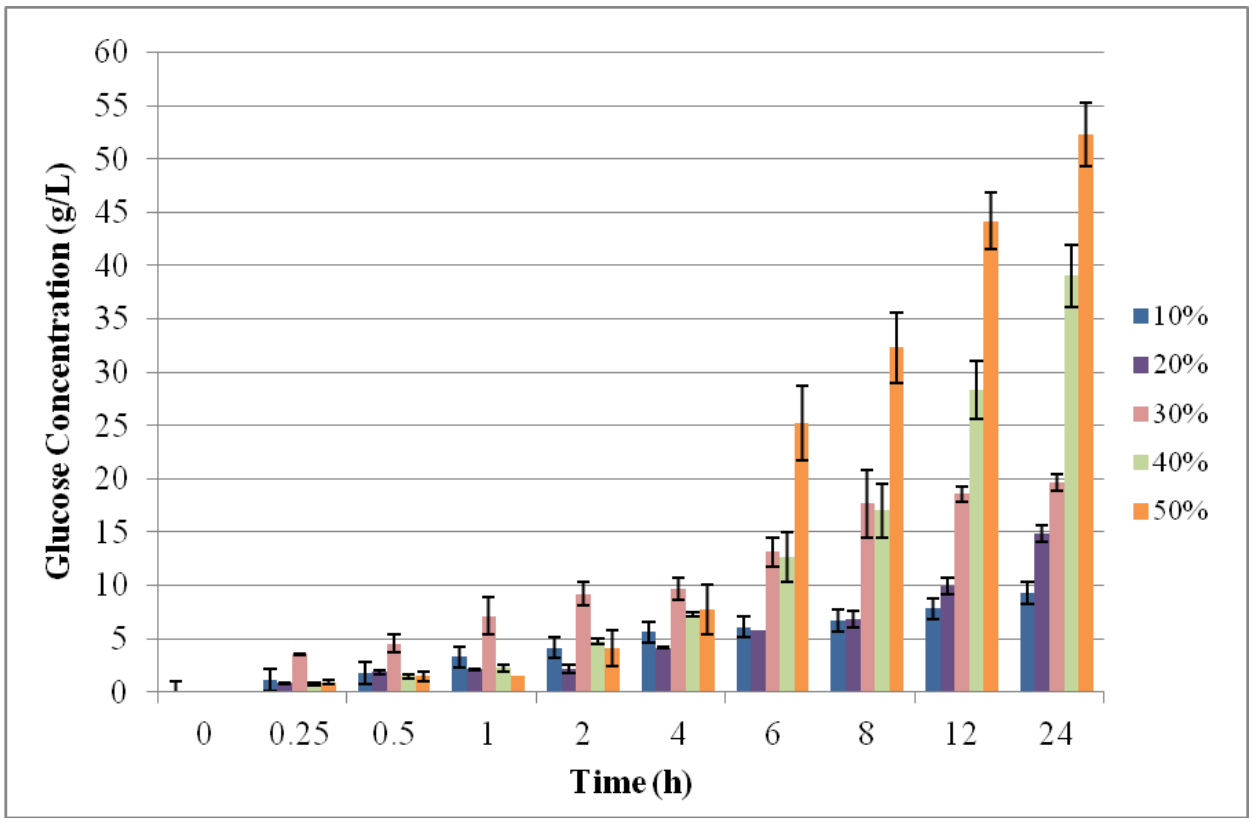


Figure 4. The effect of substrate loading on saccharification. The food waste loadings studied were 10, 20, 30, 40 and 50% (w/v) using glucoamylase loading of 2U/g substrate for 24 hours at 60^{o}C for 24 hours.

2.2.2. The effects of glucoamylase loading

The enzyme loading is another important parameter to improve the hydrolysis and speed up the reaction rate ~~up~~. To determine its effects, experiments were set up using glucoamylase loadings of 5 and 10U/g FW.

The activity level of the in-situ produced enzyme extract was not sufficient for treating the suspensions containing 50% (w/v) food waste, hence the experiments was conducted with a waste loading range of 10-40%. Figure 5 represents the glucose concentrations obtained using 5U/g FW and waste loading of in the range of 10-40% substrate loading. Although almost complete saccharification was achieved using 10 and 20% food waste concentration within 12h, it was completed at t=24h using 30 and 40% waste loading (Figure 5). The hydrolysis rates on 30 and 40% waste suspensions was lower until t=2h, possibly related to elongated gelatinization process, and it is increased dramatically later on. The gelatinization process using 5U/g FW took shorter than that of 2U/g FW, which is another advantage of using higher enzyme dosages.

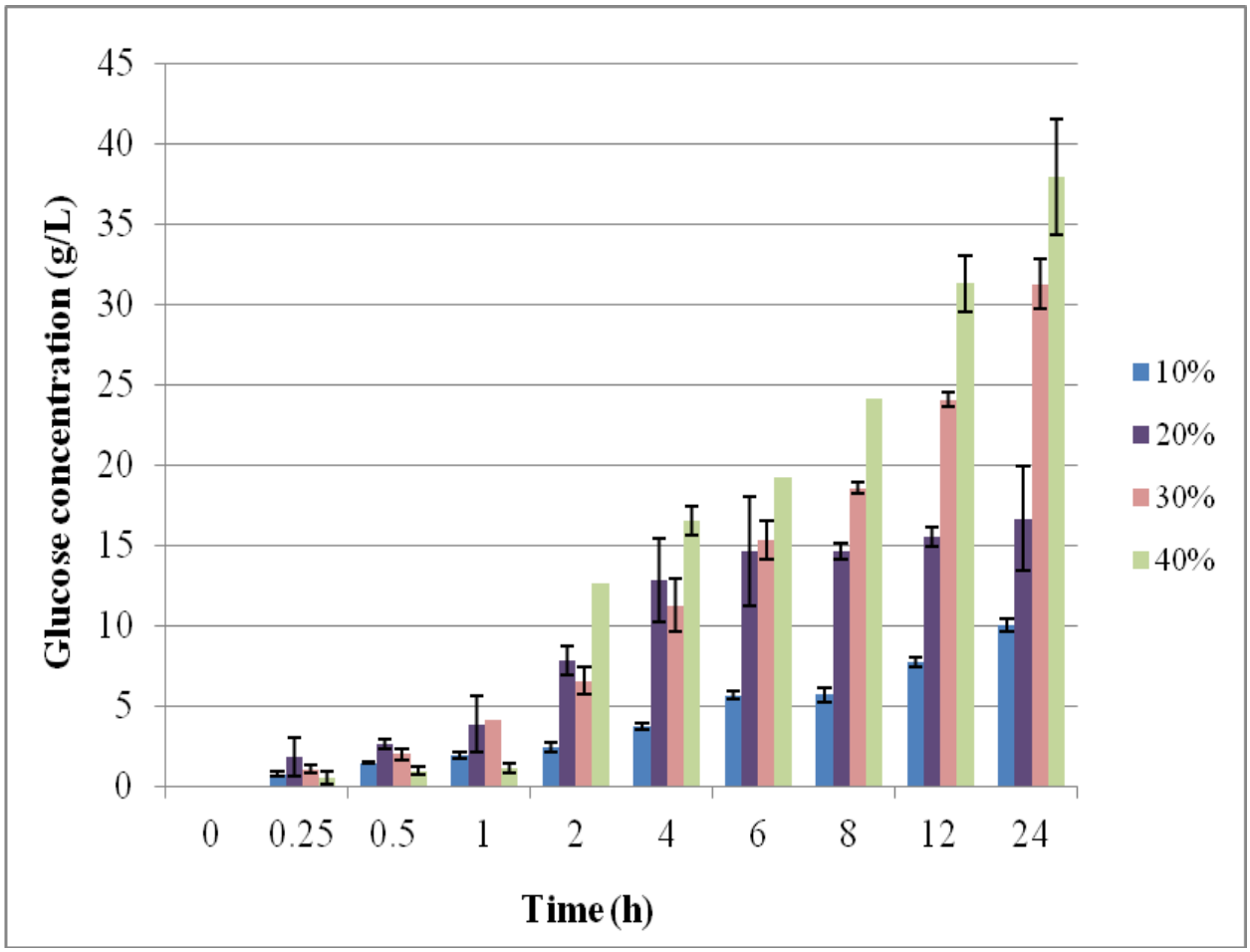


Figure 5. The effect of substrate loading on saccharification. The food waste loadings studied were 10, 20, 30, 40 and 50% (w/v) using glucoamylase loading of 5U/g substrate for 24 hours at 60^{o}C for 24 hours.

The experiments using 10U/g FW was conducted using crude enzyme cake instead of using extracted enzyme solution as the latter provides lower enzyme activity due to the dilution. The crude enzyme cake contains the enzymes, substrate (cake waste) residues and the fungal biomass. It was directly transferred to the food waste suspension (without an extraction step). The glucose production using 10U/g FW glucoamylase loading was conducted using 10, 20 and 30% waste loading for 24h. Glucose production was improved and reached to 28.9±1.44, 51.7±1.8 and 86.9±1.8 using the suspensions containing 10, 20 and 30% waste loadings, respectively (Figure 6). It is clear that these tremendous increases in glucose concentration are related to the increased starch content of the suspension, which is coming from the crude enzyme cake.

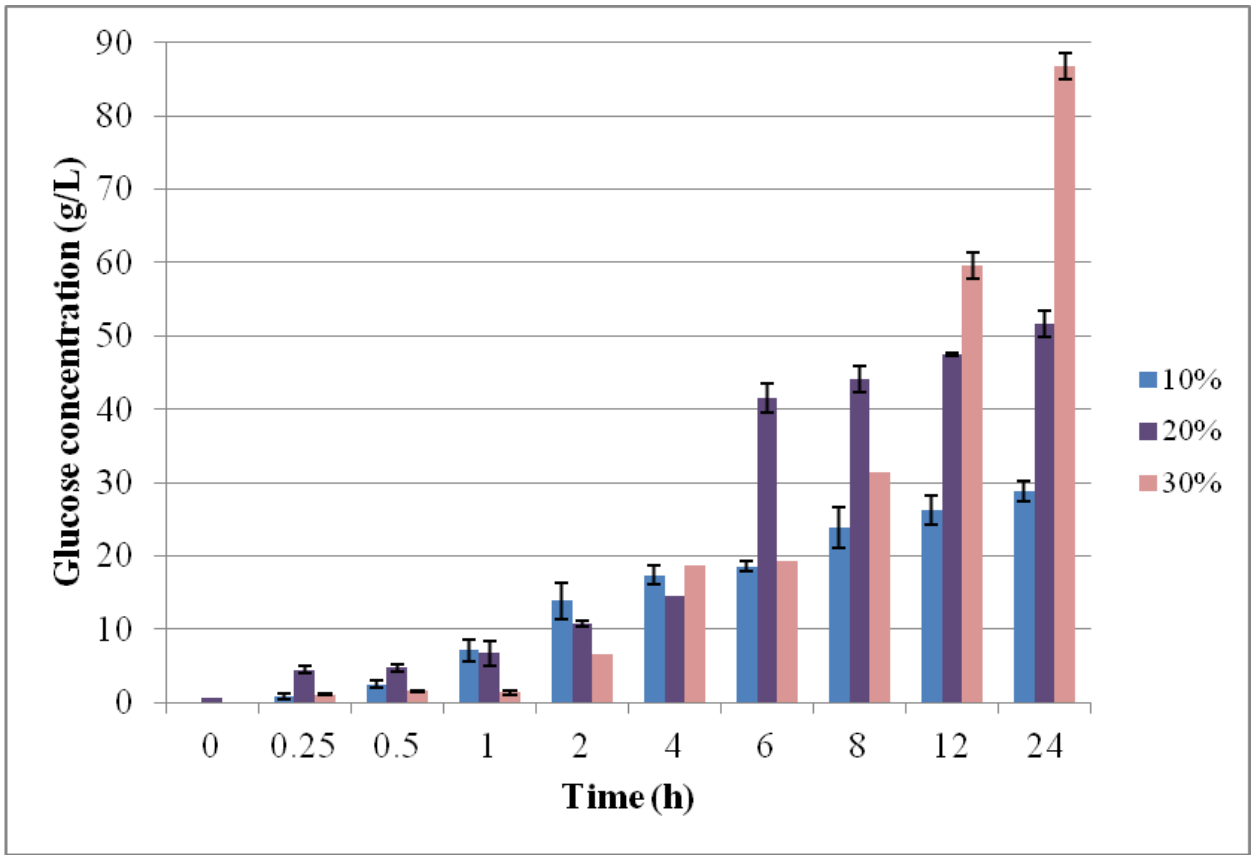


Figure 6. The effect of substrate loading on saccharification. The food waste loadings studied were 10, 20, 30, 40 and 50% (w/v) using glucoamylase loading of 10U/g substrate for 24 hours at 60ºC for 24 hours.

2.2.3. Volume reduction of food waste after enzymatic hydrolysis

In this part of the study, the effect of in-situ produced enzyme solution on the solubility i.e. volume reduction of solid food waste was investigated and evaluated. For this, Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) analyses were conducted at the end of the 24 hour-enzymatic hydrolysis. Non-hydrolyzed food waste suspension normally contained 49.35±1.75 g/L TSS with a 49.08±1.68 g/L VSS, while the pretreated food waste suspensions contained less than half of these values (Table 3). In other words, 51.1 to 62.4% of the solid residue was solubilized after the hydrolysis. By using in-situ produced enzyme solution not only the efficient hydrolysis of the starch polymer but also the food waste's volume reduction was achieved.

Table 3. The effect of enzymatic hydrolysis on VSS & TSS contents and VSS reduction (%)

Conditions	VSS (g/L)	TSS (g/L)	VSS reduction (%)
FW (no hydrolysis)	49.08±1.68	49.35±1.75	
10% FW suspension with 2U/g FW GA	24.00±0.60	24.33±0.58	51.10±1.22
10% FW suspension with 5U/g FW GA	21.48±3.33	22.08±3.58	56.24±6.78
10% FW suspension with 10U/g FW GA	20.10±5.70	22.78±3.33	59.04±11.62
20% FW suspension with 2U/g FW GA	19.30±0.80	19.63±0.93	60.67±1.63
20% FW suspension with 5U/g FW GA	18.48±0.43	26.10±2.85	62.35±0.87

2. Plans for the Next 6 and 12 Months Period

Next 6 Months:

- The optimization of food waste saccharification will be completed. For this, the enzyme loading, solid loading, temperature, pH and hydrolysis time will be optimized in two hydrolytic bioreactors.
- The effect of different pretreatments, such as heat, alkaline, microwave and ultrasonication on food waste, will be investigated to improve enzyme production and saccharification.
- The immobilization of in-situ produced enzyme solution will be studied to improve its stability and shelf life.

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 and solids destruction.
- 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. Green colored milestones shows the completed tasks while the blue ones are related to the tasks in progress.

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								

3. PERFORMANCE INDICATORS

Items	Target	Achieved
Numbers of patents or intellectual properties	1	0
Numbers of researchers*	1	1
Numbers of research man-months*	24	12
Numbers of publications in leading journals	2-3	3

*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 submitted three articles:

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

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

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

We have also submitted one Technical Disclosure on enzyme production from food waste for sludge and wastewater treatment.

Until now, 1 PhD student and 4 undergraduate students were trained.

References

Khuri, A.I., Cornell, J.A. 1987. Response Surfaces: Design and Analysis. Marcel Dekker, New York.

Melikoglu, M., Lin, C.S.K., Webb, C. 2013a. Kinetic studies on the multi-enzyme solution produced via solid state fermentation of waste bread by *Aspergillus awamori*. Biochemical Engineering Journal, **80**, 76-82.

Melikoglu, M., Lin, C.S.K., Webb, C. 2013b. Stepwise optimisation of enzyme production in solid state fermentation of waste bread pieces. Food and Bioproducts Processing.

Muthukumar, M., Mohan, D., Rajendran, M. 2003. Optimization of mix proportions of mineral aggregates using Box Behnken design of experiments. Cem. Concr. Compos. , **25**, 751-758.

Ruiz, H.A., Rodriguez-Jasso, R.M., Rodriguez, R., Contreras-Esquivel, J.C., Aguilar, C.N. 2012. Pectinase production from lemon peel pomace as support and carbon source in solid state fermentation column-tray bioreactor. Biochemical Engineering Journal, **65**, 90-95

Soni, S.K., Kaur, A., Gupta, J.K. 2003. A solid-state fermentation based bacterial alpha-amylase, fungal glucoamylase system, its suitability for hydrolysis of wheat starch. Process Biochem., **39**, 158-192.

Wang, R., Godoy, L.C., Shaarani, S.M., Melikoglu, M., Koutinas, A., Webb, C. 2009. Improving wheat flour hydrolysis by an enzyme mixture from solid state fungal fermentation. Enzyme Microb. Technol. , **44**(4), 223-228.

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.*