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 Tan**

Project Period (Please tick the appropriate box)

X July Progress Report for the period *<12/2013>* to *<07/2014>*

☐ 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 2014, we have completed the optimization of in-house enzyme production. The saccharification of food wastes from cafeteria was investigated with in-situ produced enzymes, while the conditions were optimized. In addition, the solid residuals after saccharification were further used for anaerobic digestion.

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 enzyme production

The production of enzyme was studied in both solid state and submerged fermentations inoculated with *Aspergillus awamori*, one of the well-known glucoamylase (GA) producing microorganisms. Compared to submerged fermentation, solid state fermentation (SSF) can significantly improve the production of glucoamylase (GA) from food waste. Therefore, SSF was used subsequently for producing enzymes. The production of GA by *Aspergillus awamori* with different kinds of food wastes (e.g. bread, cake, savory, vegetable, fruit, potato and mixed type food waste (MFW) from a cafeteria) was investigated (Figure 1). To analyze the effect of food waste type on enzyme production, the compositions of various types of food

wastes were determined (Table 1). 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 bread and cake wastes had higher starch content, which eventually might favor better microbial growth and amylolytic enzyme production during fermentation.

Figure 1. Solid state fermentation for enzyme production. a) Different FWs after fungal inoculation on day 0; b) Fungal micelles on cake wastes on day 4.

Table 1. Compositions of different kinds of 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
MFW (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

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. Data points show the averages from duplicate analyses.

The incubation time is related to the characteristics of the substrate, inoculum and microbial growth. Enzyme activity was mainly affected by the characteristics and homogeneity of the food waste (FW). Maximum GA production was normally achieved after 2-5 days of incubation in solid state cultures with bacteria and fungi (Melikoglu et al., 2013b; Soni et al., 2003). In this study, the maximum activity of GA was obtained with waste cakes after 4-day fermentation (Figure 2). Cake waste has a more balanced composition with high reducing sugar and protein content, thus the highest GA activity of 85.1±6.8 U/gds was obtained. Therefore, the following experiments were then conducted using cake waste as primary substrate.

Figure 3 shows that particle size of FW had a significant effect on GA production in solid state fermentation. The highest GA activity was obtained at a particle size of 0.6 mm≤X≤1.18 mm. In solid state fermentations, smaller particles can provide larger contact area for reaction. However, reduced particle size would in turn lead to increased packing density, and subsequently causing reduction in microbial growth and enzyme production (Ruiz et al., 2012). Therefore, an optimum for particle size would exist. As the highest GA activity was obtained at a particle size of 0.6≤X≤1.18, in the following experiments, size of FW was controlled in a similar range.

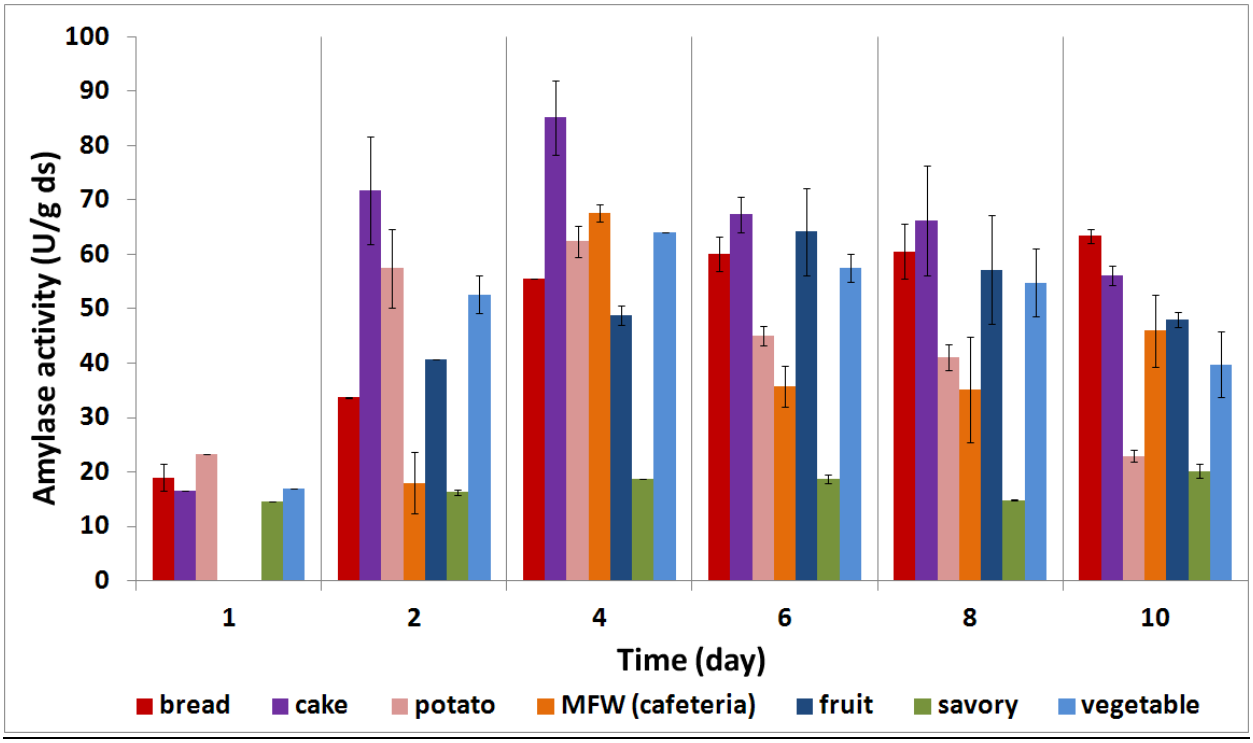

Figure 2. Effect of substrate on GA production using moisture content of 70% (wb), inoculum loading of 10^6/g substrate at neutral initial pH and 30°C. Data points show the averages from triplicate analyses.

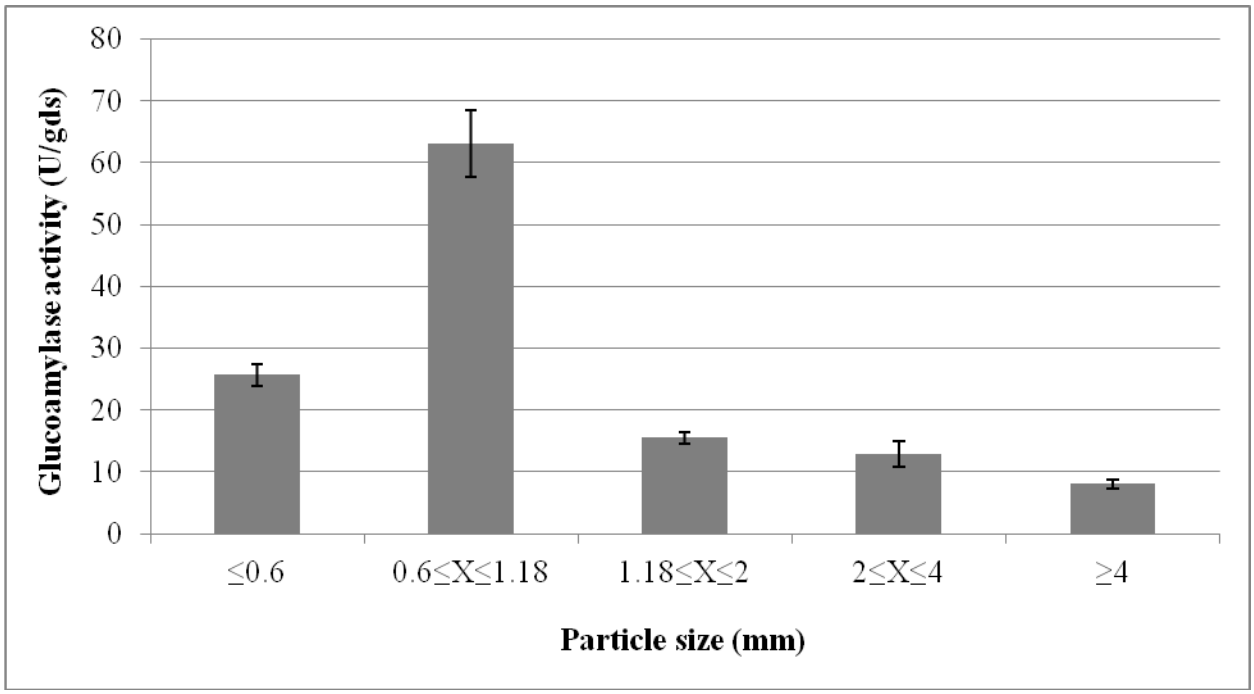

Figure 3. Effect of cake particle size on GA production using moisture content of 70% (wb), inoculum loading of 10^6/g substrate at neutral initial pH and 30°C for 6 days. Data points show the averages from duplicate analyses.

Response Surface Methodology (RSM) was also employed to optimize operation parameters (initial moisture content, inoculum loadings, initial pH and duration) for GA production from cake waste. The roles of each variable, their interactions in fermentation were analyzed with a quadratic model. Under the optimal conditions (moisture content of 69.6%, initial pH of 7.9, inoculum loading of $5.2*10^5$/g and incubation time of 6 days), 108.47 U/gds GA activity was obtained, which is 1.4 fold of the yield obtained with cake wastes at 6^{th} day of the fermentation without optimization.

2.2. Saccharification of FWs with the produced enzymes

2.2.1. Optimization of saccharification

Many factors may affect enzymatic hydrolysis including the temperature, enzyme dose, substrate concentration and the duration. The effect of reaction temperatures on hydrolysis of domestic FW (10% w/v) using in-situ produced GA was evaluated in the temperature range of 50°C and 90°C (Figure 4). During the first 6 hours, the glucose production was the highest at 70°C (6.59 g/L), and then it slowed down. After 6 hours, the glucose production at 50°C and 60°C became higher than that at 70°C. This might be because of enzyme denaturation at temperatures higher than 60°C. These findings are similar to the results reported in the literature. Melikoglu et al. (2013a) evaluated the kinetics of the GA using the same microorganism and found that the maximum enzyme activity was achieved at 60°C and then tended to decline at higher temperatures, due to thermal deactivation of the enzyme. The highest glucose concentration of 10.4 g/L corresponding to a saccharification degree of 97.9% was obtained at 60°C after 24 hours. Hence, the following studies were conducted at 60°C for 24 hours.

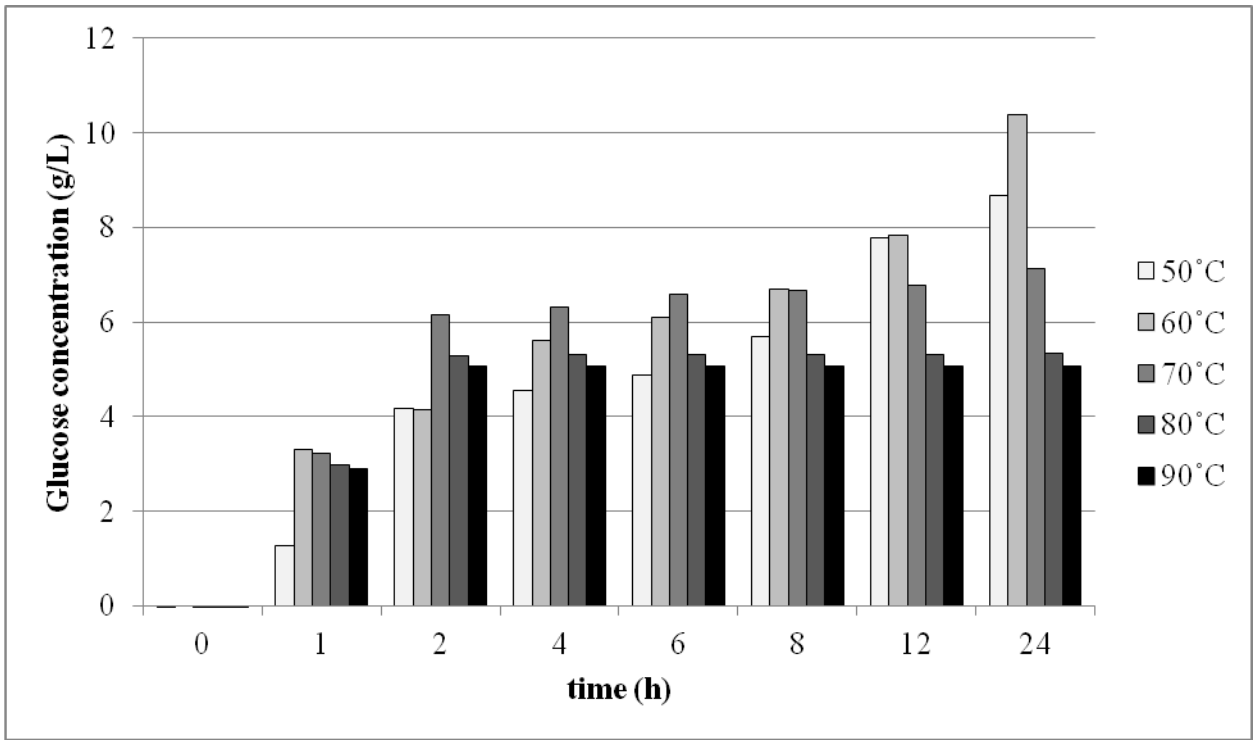

Figure 4. Effect of temperature on glucose formation during the hydrolysis of domestic FW with the produced GA preparation. Data points show the averages from duplicate analyses.

To study the effect of FW concentration on the enzymatic hydrolysis, GA treatment was conducted at 10, 20, 30, 40 and 50% (w/v) FW loadings with 2U/g FW enzyme loading for 24 hours. The waste concentrations higher than 50% were not studied due to high viscosity of the suspension, which certainly would inhibit enzyme activity. The glucose released increased dramatically with an increase in substrate loading, while the hydrolysis continued until 24th hour (Figure 5). The hydrolysis rate was sluggish at the waste loadings of 30, 40 and 50% during the first 4 hours and increased dramatically afterwards. This might be related to elongated gelatinization of starch. At t=24 hour, the maximum glucose concentrations 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 were obtained at the respective FW loadings of 10, 20, 30, 40 and 50% (w/v). Moreover, the saccharification degree at t=24h reached 99.8% at the FW loading of 50%. In these experiments, no substrate inhibition was observed, i.e. no dilution of FW would be needed in the loading range studied, which would help to reduce generation of wastewater. It should be noted that even at t=12h, the saccharification degree was found to be 55-88%, depending on the waste concentration.

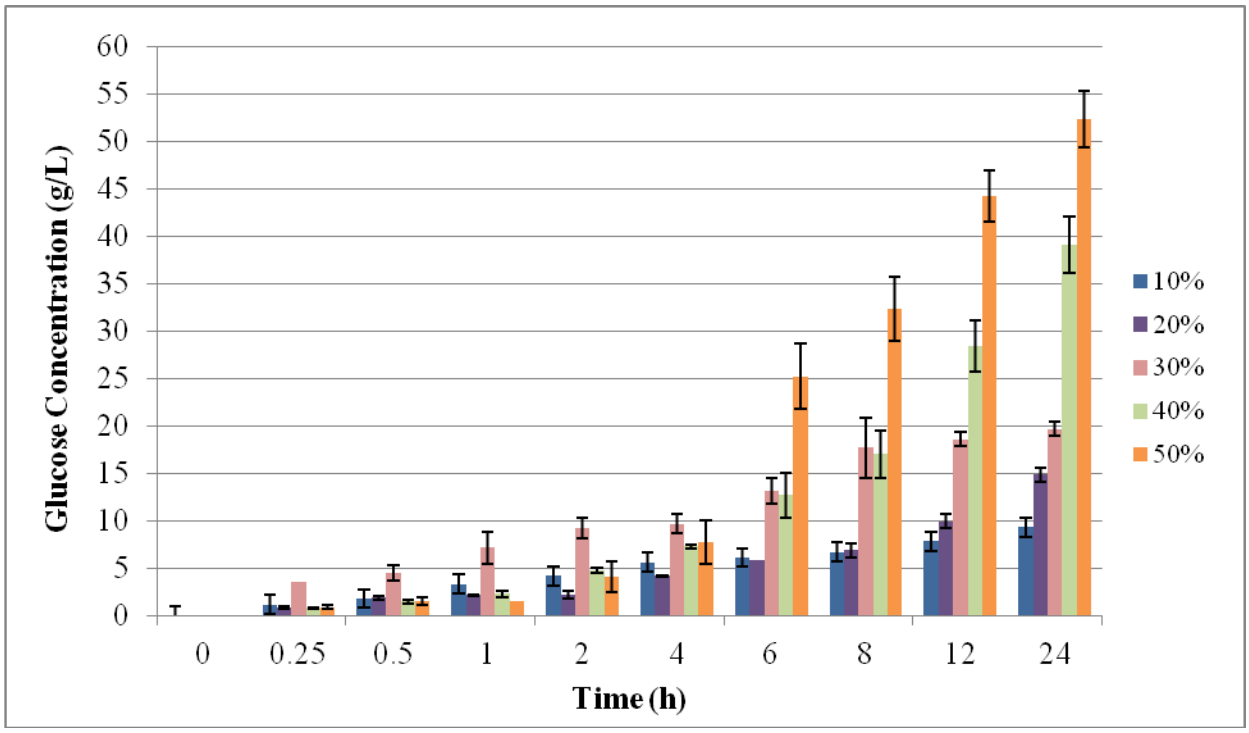

Figure 5. The effect of substrate loading on saccharification. The FW loadings studied were 10, 20, 30, 40 and 50% (w/v) using GA loading of 2 U/g substrate for 24 hours at 60°C for 24 hours. Data points show the averages from duplicate analyses.

As maximizing production of glucose is the main target of this study, saccharification at higher enzyme loadings should need to be investigated for increasing glucose concentration, while shortening hydrolysis time. For this purpose, experiments were carried out at two different GA loadings of 5 and 10 U/g FW, respectively. The activity level of the in-situ produced enzyme extract was not high enough for treating the suspensions of 50% (w/v) FW; hence the experiments were conducted in a waste loading range of 10-40%. Figure 6 shows the glucose concentrations obtained at 5 U/g FW and 10-40% of waste loadings. Almost complete saccharification was achieved within 12 hours at 10 and 20% FW loadings, while 24 hours were needed at the waste loading of 30 and 40% (Figure 6). The hydrolysis rates of 30 and 40% waste suspensions were lower until t=2h, possibly due to elongated gelatinization. Afterwards, it increased quickly. The gelatinization process at 5U/g FW was found to be much shorter than that at 2U/g FW, showing advantage of using higher enzyme dosages.

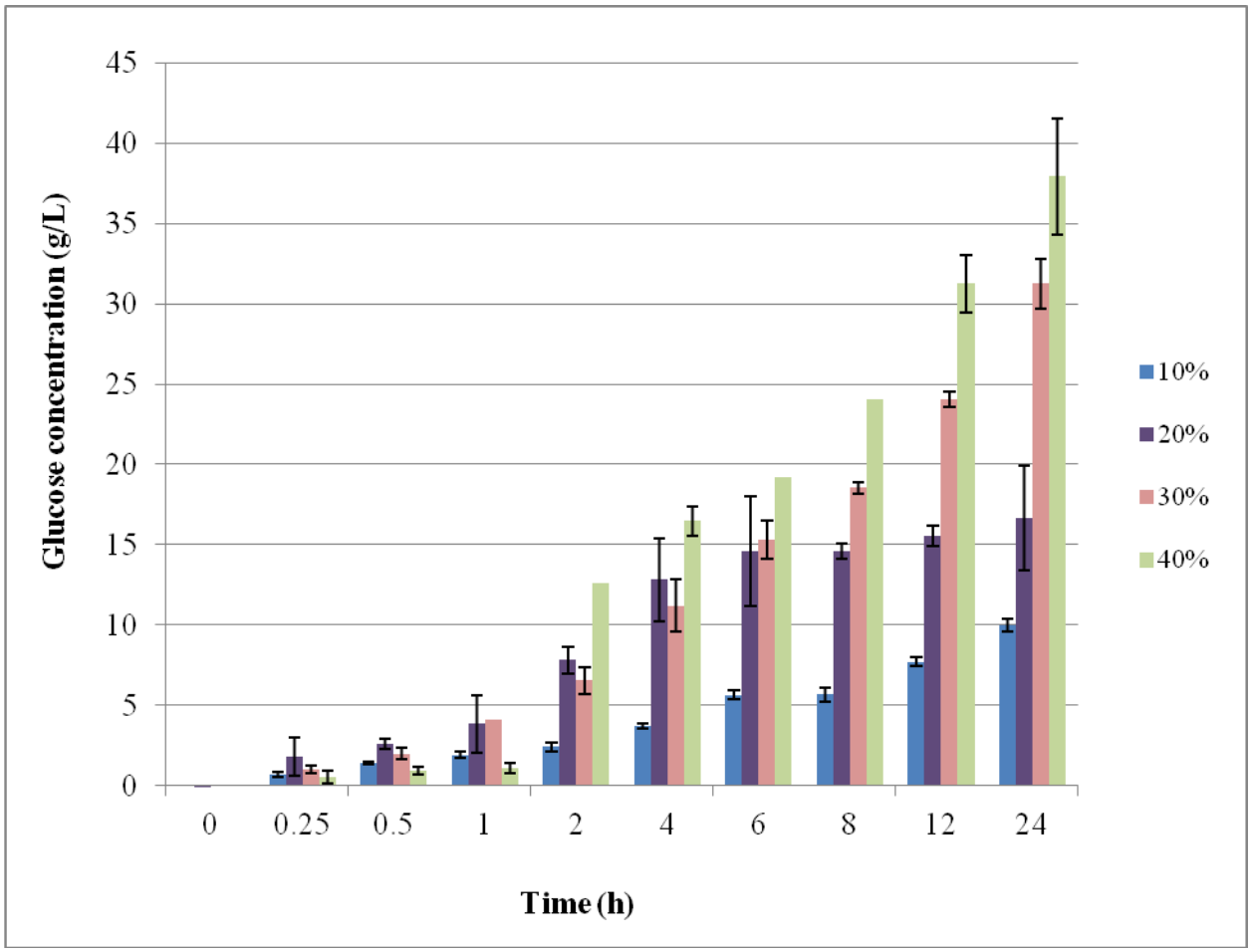

Figure 6. The effect of substrate loading on saccharification. The FW loadings studied were 10, 20, 30, 40 (w/v) using GA loading of 5U/g substrate for 24 hours at 60°C for 24 hours. Data points show the averages from duplicate analyses.

The tests using 2U/g FW and 5U/g FW were conducted using extracted enzyme. The experimental sets using 5U/g FW for 50% FW loading and the experiments using 10U/g FW were not possible due to the dilution of enzyme by the extraction. Therefore, these experiments were conducted using crude enzyme-cake instead of extracted enzyme solution as it has lower enzyme activity due to the dilution with water. The crude enzyme-cake contains the enzymes, substrate (cake waste) residues and the fungal biomass, and was directly added to the FW suspension, without further extraction. The glucose production with10 U GA/g FW was conducted at the waste loadings of 10, 20 and 30% for 24 h. It was found that glucose production was improved and reached 28.9±1.44, 51.7±1.8 and 86.9±1.8 g/L at the waste loadings of 10, 20 and 30%, respectively (Figure 7). Such significant improvement in glucose production would be related to a greater starch content of the suspension, which results from the crude enzyme cake.

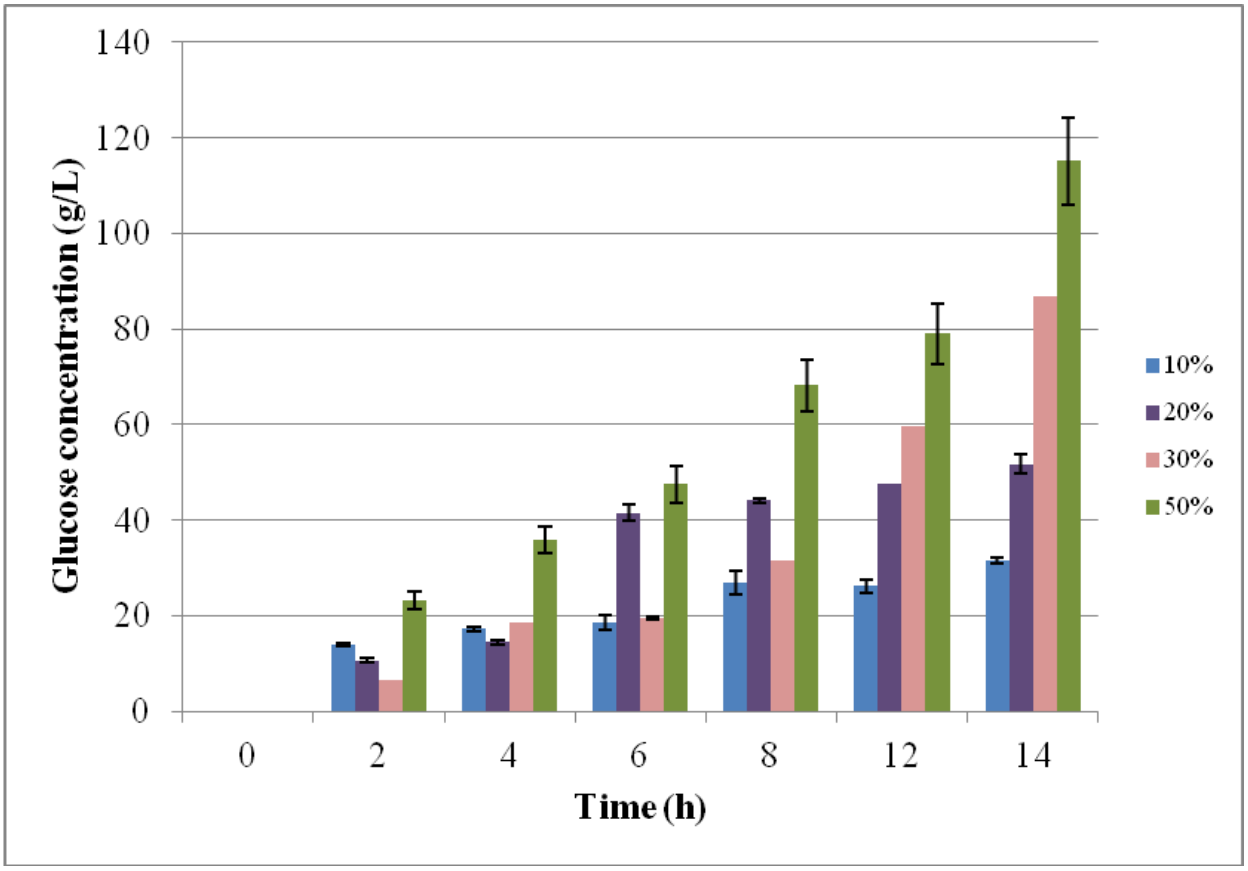

Figure 7. The effect of substrate loading on saccharification. The FW loadings studied were 10, 20, 30 and 50% (w/v) using GA loading of 10U/g substrate at 60°C for 24 hours. Data points show the averages from duplicate analyses.

2.2.2. Volume reduction of FW after enzymatic hydrolysis

In this part of the study, the effect of in-situ produced enzyme solution on the solubilization of FW, i.e. volume reduction of solid FW was investigated. And Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were determined at the end of the 24 hour-enzymatic hydrolysis. Initial FW suspension contained 49.35±1.75 g/L TSS with a 49.08±1.68 g/L VSS (Table 2). After hydrolysis, 51.1 to 62.4% of the FW was solubilized. The in-situ produced enzyme solution can significantly improve hydrolysis of the starch polymer, but also help to reduce the volume of FW.

Table 2. The effect of enzymatic hydrolysis on VSS & TSS contents and VSS reduction (%).Data points show the averages from duplicate analyses.

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

2.2.3. Utilization of different crude enzymes for FW saccharification

FW contains some other carbohydrates like cellulose and hemicellulose other than starch. Therefore, the addition of cellulases and hemicellulases might further improve the final glucose concentration. For this reason, a fungus (*Trichoderma reesei*) was used to produce crude enzyme for high activity cellulase production.

Various agricultural and kitchen waste residues were assessed for their ability to support the production of cellulase by *Trichoderma reesei* in solid state fermentation. Different FWs such as banana peel, soybean flour, potato peels, oat meal and orange waste were used as substrate to produce cellulases as the highest cellulase activities were reported using these substrates in the literature. The substrates simply moistened with water (to a 70% final moisture content), were found to be well suited for fungal growth, producing good amounts of cellulases after 96 h without the supplementation of additional nutritional sources. The highest cellulase activity (12.2 FPU/mL) was obtained using oat meal (Figure 8).

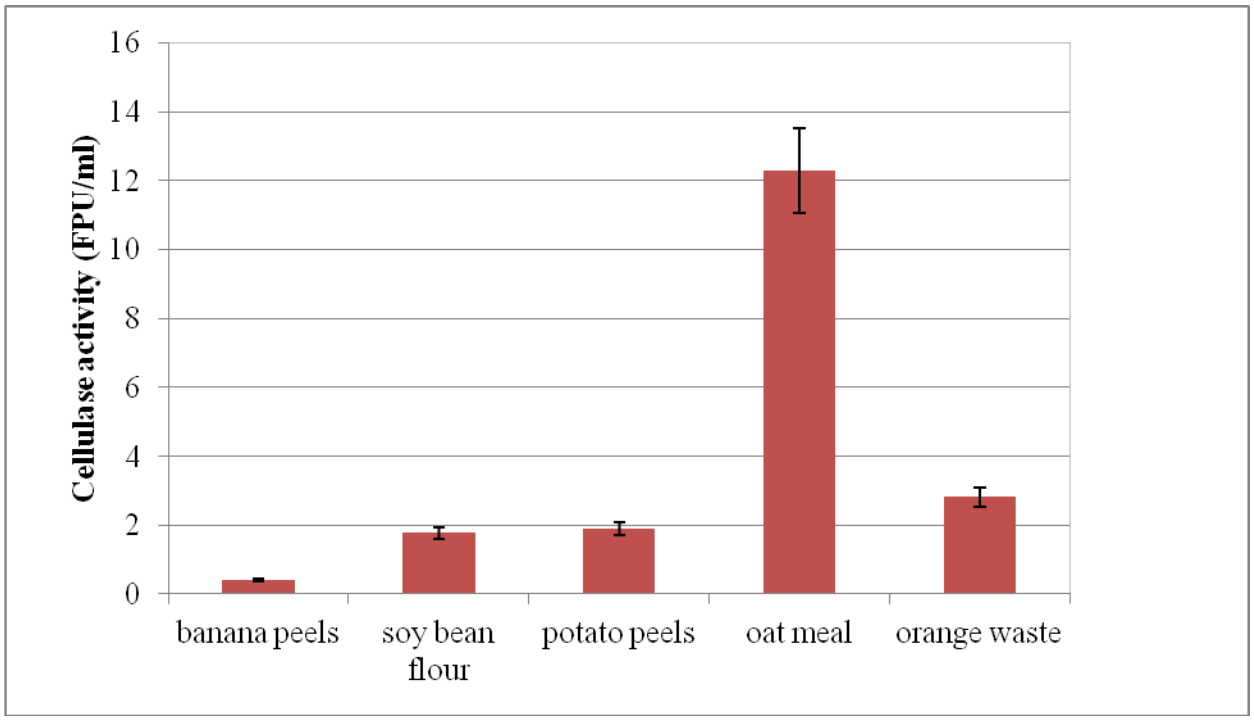

Figure 8. The effect of different substrates on cellulase production using *T.reesei* using SSF at 25ºC, 6 days. Data points show the averages from duplicate analyses.

The effect of crude enzymes rich in GA and cellulase were evaluated. GA rich fungal enzyme cocktail resulted in 115 g/L glucose after 24 h hydrolysis, while only 36.5 g/L glucose can be achieved using cellulase rich enzyme cocktail (Figure 9). Even though the enzymatic hydrolysis using GA rich fungal enzymes cocktail resulted in higher glucose production compared to cellulase rich one, 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, 140.1 g/L glucose was produced.

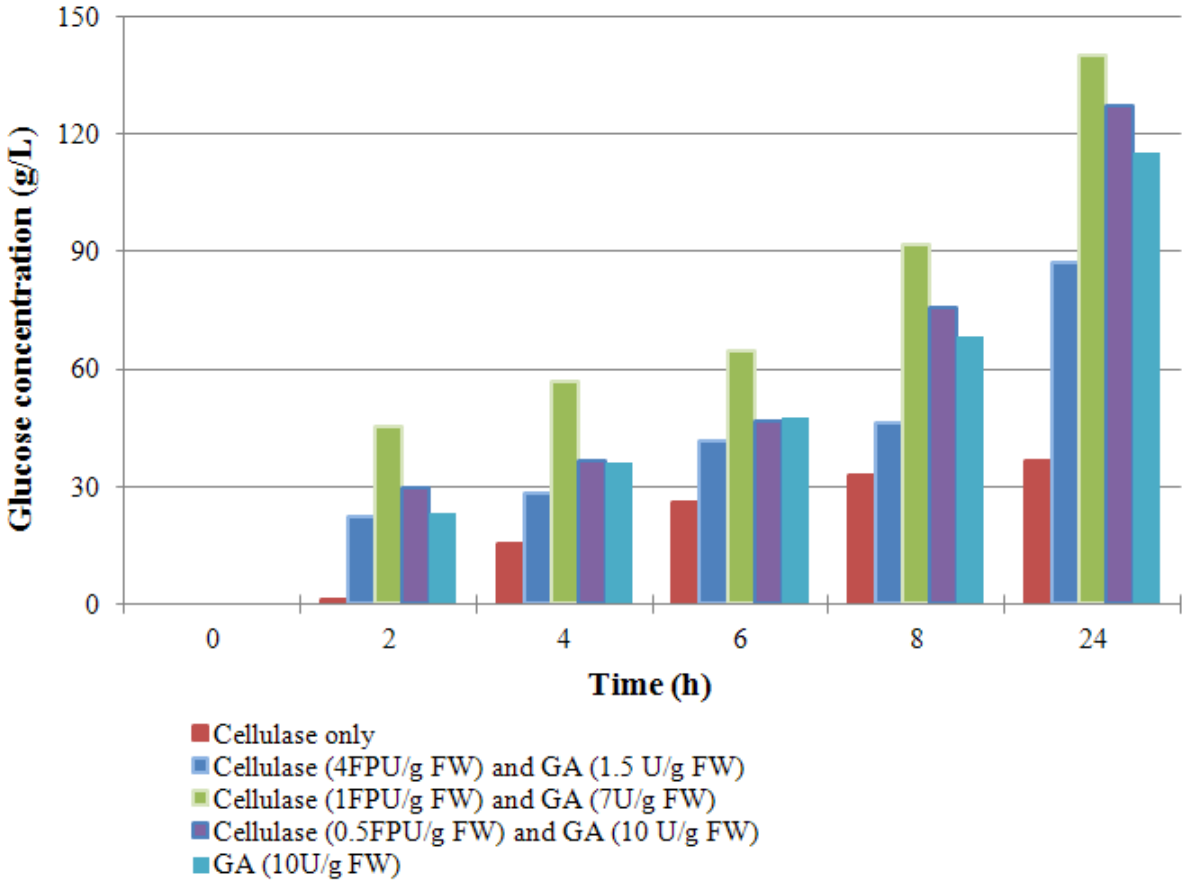

Figure 9. The effect of GA and cellulase rich enzymes cocktails on glucose production using FW loading of 50%, at 60^{o}C for 24 hours. Data points show the averages from duplicate analyses.

2.2.4. <u>An integrated process for FW management</u>

Figure 10 presents the graphical abstract of the study. Using fungal enzymes cocktail, FW can be converted to glucose (140 g/L) and Free Amino Nitrogen (FAN) (2.5-4.2 g/L), which provide a well balanced potential fermentation feedstock that can be used to produce many high value products. Although direct biogas production from raw FW may not be an economically attractive option, the waste solid remained after enzymatic hydrolysis can still be used for anaerobic digestion to improve the efficiency of the whole process. A methane yield of 40 mL/g dry solid was obtained vis-à-vis 190 mL CH_4/g dry solid obtained from untreated FW anaerobically.

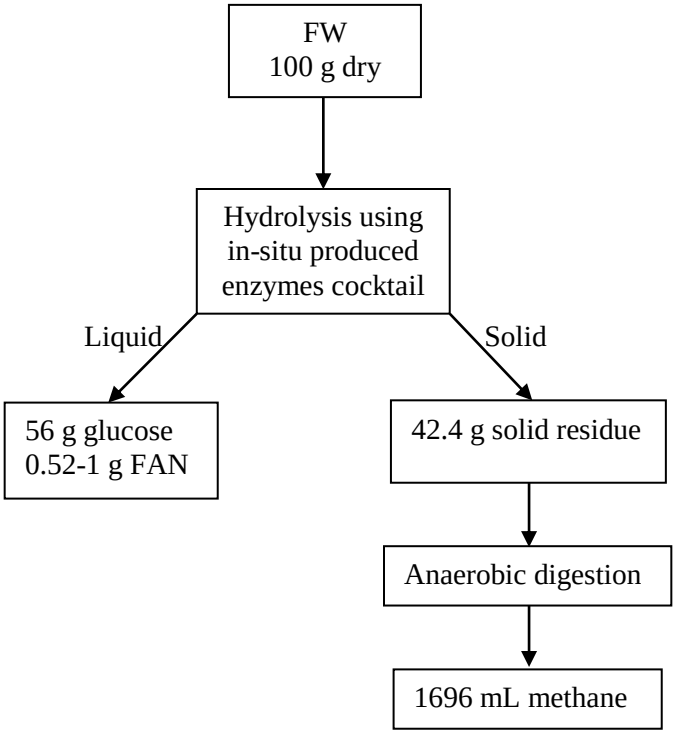

Figure 1. Glucose and biomethane recovered from 100 g dry FW.

Table 3 presents the potential amounts of the products can be generated and the price of the products using the glucose and FAN rich FW hydrolyzate. The amounts of products can be obtained from 1 ton FW are also presented. As can be seen, the market prices of liquid fuels and platform chemicals are higher than that of methane. Meanwhile, the market prices of platform chemicals are much higher than that of fuels. Therefore, more studies should be conducted to reduce the cost of fuel and/or platform chemical production process costs.

Table 3. Some high value products that can be produced using FW hydrolysis and their market prices.

Product	**Yield**	**Quantity/ ton FW**	**Price (USD)**	**USD/ ton FW**	**References**
Methane	546 mL/g VS	152.9 L	4-6/million metric BTU	0.011	(Indexmundi, 2014; Uçkun Kiran et al., 2014)
Ethanol	0.49 g/g glucose	173.9 L	2.21/gallon	101.4	(Uçkun Kiran et al., 2014; Wikipedia, 2014)
Butanol	0.19 g/g glucose	53.2 L	3.75/gallon	52.6	(Bankar et al.; Wikipedia, 2014)
Lactic acid	1.29 g/g glucose	361.2 kg	1300-1600/ton	469.6	(Sakai & Yamanami, 2006; Wee et al., 2006)
Citric acid	0.80 g/g glucose	224 kg	1300-1600/ton	291.2	(Hamdy, 2013; Shojaosadati & Babaeipour, 2002)
Succinic acid	1.16 g/g glucose	324.8 kg	3000-5000/ton	974.4	(Leung et al., 2012; RCS, 2014)
PHA	0.44 g/g glucose	123.2 kg	4960-6062/ton	611.1	(PlasticsEngineeringBlog, 2014; Xu et al., 2010)

3. Discussion

Current waste management strategies for food waste (FW) have been facing more and more challenges with strong environmental concerns. Nowadays, landfilling of organic waste still remains the most economic option for waste management (Tatsi & Zouboulis, 2002). However, uncontrolled releases of biogas and leachates may lead to serious environmental problems (Abu-Rukah & Al-Kofahi, 2001). Singapore has practiced incineration of FW for years, and the incineration ashes are disposed off in the Semakau offshore landfill which would be saturated in next 20-30 years due to rapidly increasing waste generation.

Composting has a relatively low environmental impact and a high economic efficiency compared to other treatment methods. However, the high moisture content of FW will lead to substantial release of leachate (Cekmecelioglu et al., 2005). Indeed, compost is more expensive than commercial fertilizers and the current market of compost produced from FW is limited (Aye & Widjaya, 2006).

Incineration is the fastest way to treat FW and the weight can be reduced by 70‑80% (Table 4) but it is not always feasible, typically due to the energy required to evaporate the large amounts of water in FW. The remaining ash, however, needs to be disposed in landfill sites. Together with this, the lack of public acceptance due to the possible generation of harmful emission makes incineration of FW unsustainable (Schumacher & Domingo, 2006).

Anaerobic digestion is another alternative which yields methane and carbon dioxide as metabolic end products and therefore could be feasible from an economic and environmental point of view because methane is used as an energy source (Othman et al., 2013). Hirai et al. (2001) evaluated the environmental impacts of FW treatment and found that utilizing a methane fermentation process prior to incineration reduces approximately 70 kg CO_2eq/ton waste of the global warming potential, due to the substitution effect. Food waste is also used as animal feed. The disadvantages are its variable composition and the high moisture content, which favors microbial contamination (Esteban et al., 2007). To prevent this, animal feed is generally dried but greenhouse gas emission increases depending on the energy usage during the drying process, which is related to the water content of FW (Takata et al., 2012).

Current waste management methods were compared considering their recycling strategies, volume reduction, process duration and final products (Table 4). One of the most important aspects is the overall weight of FW that can be reduced. Dry weight reductions of 80%, 66% and 43% were reported for anaerobic digestion, enzymatic hydrolysis using commercial enzymes and composting, respectively, while enzymatic hydrolysis using fungal enzymes cocktail is more advantageous in terms of weight reduction. More than 80% of the initial solid dry weight was reduced by enzymatic hydrolysis using fungal enzymes cocktail. A sustainable FW treatment process should efficiently reduce and allow a recycling of the organic matter. The conventional treatment and recycling strategies are based on conversions of waste into energy/heat and soil. Contrarily, enzymatic hydrolysis of FW using fungal enzymes cocktail provides the opportunity to hydrolyze FW. This facilitates the use of the obtained hydrolysate as fermentation feedstock for the production of high-value compounds such as platform chemicals and biofuels (Table 3). This is economically more advantageous than current waste management strategies (Tuck et al., 2012). Another advantage of FW hydrolysis using fungal enzymes cocktail is that it does not lead to any formation of contaminants affecting human health. The remaining solids from FW hydrolysis can be used as feedstock in methane production.

Table 4. Various FW treatment processes.

Process	Dry weight reduction, duration	Products	Recycling strategy
Disposal in landfill sites [a]	Years	Methane, leachate	Waste to soil
Composting [b, c, d, e]	43%, weeks to months	Fertilizers	Waste to soil
Anaerobic digestion [b, f, g]	80%, weeks	Methane, fertilizers	Waste to energy/heat and soil
Incineration [b, h, i]	70-80%, minutes	Heat, ash	Waste to energy/heat and soil
Enzymatic hydrolysis using commercial enzymes [j]	66%, hours	Sugar monomers, fertilizer	Waste to high value products, feed, soil and energy/heat
Enzymatic hydrolysis using fungal enzymes cocktail	80-90%, days	Sugar monomers, amino acids, fertilizer	Waste to high value products, feed, soil and energy/heat

[a] Kjeldsen et al. (2002), [b] Arvanitoyannis et al. (2008), [c] Adhikari et al. (2009), [d] Zhang and Jahng (2012), [e] Seo et al. (2004), [f] Kim et al. (2011), [g] Zhang et al. (2007), [h] Pirotta et al. (2013), [i] Cherubini et al. (2009), [j] Lam et al. (2013).

2. Plans for the Next 6 Months Period

- 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 FW by *Aspergillus*: characterization of enzymes								
✓ Milestone 4: Solid State fermentation of FW 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	2 TD
Numbers of researchers*	1	1
Numbers of research man-months*	24	18
Numbers of publications in leading journals	2-3	5

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

We have already prepared five articles and two Technical disclosures:

Journal Articles:

1. Uçkun Kiran, E., Trzcinski, A. P., Ng, W.J., Liu Y. 2014. Bioconversion of food waste to energy: a review, Fuel (134) 389-399.
2. Uçkun Kiran, E., Trzcinski, A. P., Ng, W.J., Liu Y. 2014. Enzyme production from food wastes using a biorefinery concept: a review, Waste and Biomass Valorization (http://link.springer.com/article/10.1007/s12649-014-9311-x).
3. Uçkun Kiran, E., Trzcinski, A. P., Liu Y. 2014. Enhanced glucoamylase production from food waste using solid state fermentation and its evaluation in the hydrolysis of domestic food waste, Waste Biomass valorization (under review).
4. Uçkun Kiran, E., Trzcinski, A. P., Liu Y. 2014. Biorefineries for chemical production from food waste, (submitted to Journal of Chemical Technology and Biotechnology).
5. Uçkun Kiran, E., Trzcinski, A. P., Liu Y. 2014. Enhancing methane production from food waste using enzymatic pretreatment, (to be submitted to Renewable Energy).

Technical disclosures:

1. Uçkun Kiran, E., Trzcinski, A. P., LiuY. 2014. Enzyme production from food waste for sludge and wastewater treatment.
2. Uçkun Kiran, E., Ong, Y.P., Trzcinski, A. P., Liu Y. 2014. Enhancing food waste saccharification by microwave pretreatment.

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.

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.*

References

Abu-Rukah, Y., Al-Kofahi, O. 2001. The assessment of the effect of landfill leachate on ground-water quality – a case study El-Akader landfill site – north Jordan. *J. Arid Environ.* , **49**, 615-630.

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