ETRP PROGRESS REPORT

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GUIDELINES FOR ETRP PROGRESS/FINAL REPORT

Project Reference No.: ETRP 1201 105	**Project Completion Date:** June 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 January Progress Report for the period <*07/2014*> to <*01/2015*>

☐ 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 January 2015, we have completed the optimization of in-house enzyme production and food waste hydrolysis studies. We also conducted the food waste hydrolysis studies in 3L bioreactors. In addition, we also started the mesophilic anaerobic digestion studies using the solid residuals remained after the hydrolysis process to recover more energy and improve waste volume reduction.

Milestones and Deliverables	Implementation Schedule										
	Year 1				Year 2				Year 3		Remarks
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	
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											
7: Mesophilic AD of residues at different solid loadings											
8: Thermophilic AD of residues at different solid loadings											
9: Use of anaerobically digested solids as soil stabilizers											
Actual implementation											

2. RESULTS & DISCUSSION

2.1 Food Waste Hydrolysis In 3L Hydrolytic Bioreactors

Mixed food waste collected from a cafeteria at Nanyang Technological University was hydrolyzed with fungal mash produced from waste cakes in 3L bioreactors. The composition of mixed food waste and waste cakes were given in Table 1.

Table 1. Composition of food wastes in one gram of dry mass.

FW	Total Carbohydrates (mg)	Starch (mg)	Protein (mg)	Lipid (mg)	Ash (mg)
Cake waste	643±12	458±30	141±8	161±8	39± 2
Mixed FW	768±52	603±38	86±4	153±21	29±2

The food waste was hydrolyzed using fungal mash rich in GA. It can be seen in Figure 1 that the glucose concentration increased with time until a plateau of 118.5±5.4 g/L after 12 h hydrolysis. Although similar concentrations (115 g/L) could be obtained in bench scale studies, the hydrolysis time required for complete hydrolysis was 24 h. The time required to complete the hydrolysis in bioreactor experiments is 12 h, which is half of the time required in previous case. This should be related to proper mixing with the agitators in bioreactors. Such a high concentration is more than enough to be used as fermentation feedstock in following fermentation studies to produce value-added products.

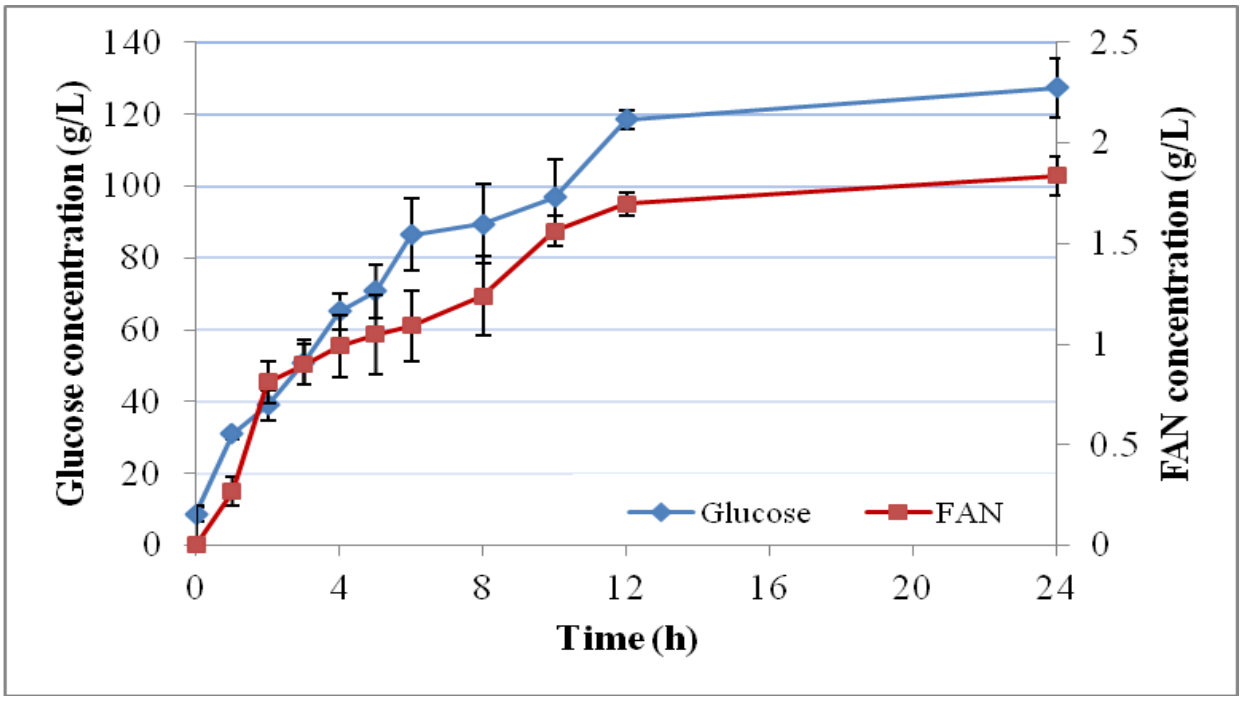

Figure 1. Effect of enzymatic pretreatment on glucose and FAN production from FW in 3L hydrolytic bioreactors.

According to the glucose concentrations obtained, about 80-92% of the initial carbohydrates in FW could be hydrolyzed by the fungal mash produced in this study. This in turn suggests that the fungal mash essentially contains multiple-enzymatic activity required for FW hydrolysis. Moreover, the fungal mash produced in this study has the advantages of no need for enzyme separation and purification, low cost and in-situ massive production, and finally high combined enzymatic activity towards FW hydrolysis.

The release of proteins during the hydrolysis of FW by the fungal mash was also determined in terms of Free Amino Nitrogen (FAN). Total FAN content in the hydrolyzates obtained during the FW pretreatment with the fungal mash increased to about 0.81 g/L after 2 hours, and then stabilized at 1.84 g/L after 24 hours. This can be explained by the the protease activity in the fungal mash at the level of 0.41 U/mL. The total nitrogen analysis further revealed that nearly 80% of proteins in FW were solubilized by the fungal mash. Moreover, proteases could also help release of carbohydrates by breaking down binding proteins (Kim et al., 2006). Hence, the release of readily soluble polysaccharides would enhance FW solubilization through the synergistic actions of the various enzymes in the fungal mash. It should be noted that high FAN concentration is essential for subsequent fermentation as it provides a balanced nitrogen source for bacterial metabolism and growth. In this study, the bio-available carbon to nitrogen (C/N) ratio was found to be 27.7 in the hydrolyzate obtained from the FW with the fungal mash pretreatment. It has been known that a feedstock with a C/N ratio greater than 30 is considered to be deficient in nitrogen for a biological treatment process (Gomez et al., 2005). Therefore, the hydrolyzate obtained from fungal mash would be a good biomedium for subsequent fermentation studies to produce value added products like biofuels and platform chemicals. It should also be noted that 54.1% of reduction in VS was reached at the end of the fungal mash pretreatment, indicating high efficiency of the fungal mash in food waste volume reduction.

In order to improve the glucose production GA and cellulase rich fungal mashes were used together in 3L hydrolytic bioreactor. The glucose concentration further increased using these two fungal mashes together (Figure 2). Using both fungal mashes together, the glucose concentration increased with time until12 h hydrolysis and reached to a plateau at 136 g/L, which is almost the final concentration (140 g/L) obtained in bottle tests at the end of the 24 h hydrolysis.

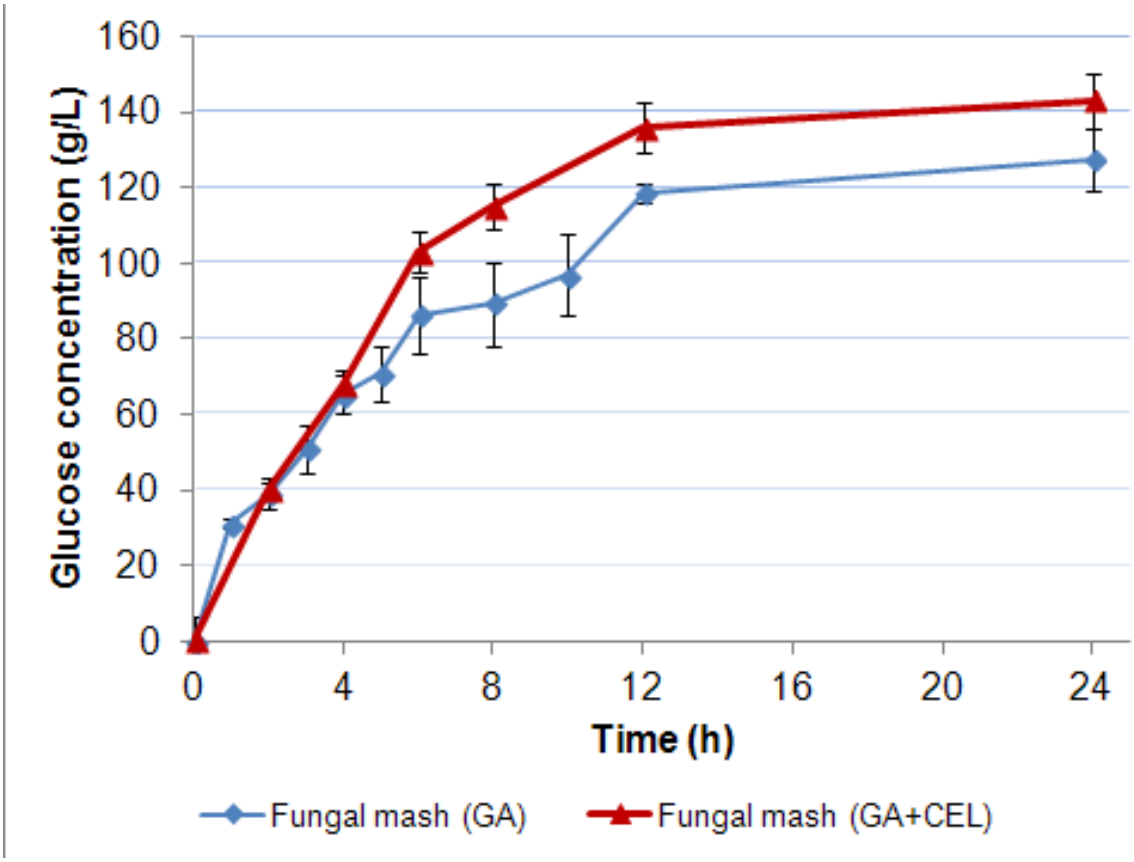

Figure 2. The effect of fungal mashes (GA and GA+Cellulase) on glucose production from food waste in 3L hydrolytic bioreactors.

The ultimate glucose concentration and the time required for the hydrolysis are mainly related to the moisture and carbohydrate content of the food waste, enzymes and substrate loadings and also the process parameters. The glucose concentration obtained by fungal mash pretreatment is higher than those reported in the previous studies with commercial enzymes (Table 2). In the study by Pleissner et al. (2014), a glucose concentration of 143 g/L was obtained after 48 h fermentation. In this study, very close glucose concentration was obtained within 12 hours, which is much more shorter than the time required in Pleissner et al. (2014).

Table 2. Glucose concentrations obtained from the hydrolysis of food waste.

Pretreatment	Duration (h)	$C_{Glucose}$ (g/L)	References
GA, protease, cellulase	12	69.8	(Kim et al., 2011)
GA, cellulase, α-amylase, β-glucosidase	6	64.7	(Uncu & Cekmecelioglu, 2011)
GA, cellulase, α-amylase, β-glucanase, xylanase, hemicellulase, arabinase	8	79.1	(Jeong et al., 2012)
GA, cellulase, α-amylase, β-glucanase, xylanase, hemicellulase, arabinase	6	44.0	(Moon et al., 2009)
GA, α-amylase, β-glucosidase	24	65.0	(Hong & Yoon, 2011)
Fungal mash (GA+protease)	48	143	(Pleissner et al., 2014)
Fungal mash (GA)	**12**	**118.5**	**This study***
Fungal mash (GA+cellulase)	**12**	**136.0**	**This study***

GA: Glucoamylase, C: concentration.

2.2 Anaeobic Digestion of the solid residues

In order to improve the volume waste reduction and recover energy, AD process was integrated to the hydrolysis system. After the hydrolysis, the medium was centrifuged at 10000 g for 10 min. The glucose rich biomedium was separated; while the solid residues were collected for anaerobic digestion. The residual solids were anaerobically digested using mesophilic secondary sludge as inoculum (Ulu Pandan).

As most of the carbohydrates and proteins hydrolyzed and remained in the liquid biomedium, solid residue is poor in nutrients. Therefore, a long lag phase was observed in methane generation until the 13th day of the AD process (Figure 3). Later on, the methane and biogas production rates increased and the cumulative biogas and methane yields reached to 658 and 256 mL/g VS at the end of the 50 days AD process, respectively. Later on, the production did not increase much most probably due to the insufficient nutrient in the medium. At the end of the AD, around 71% mass reduction was obtained. Considering 54% mass reduction in hydrolysis step, total 86% mass reduction was obtained in this integrated process (hydrolysis+AD).

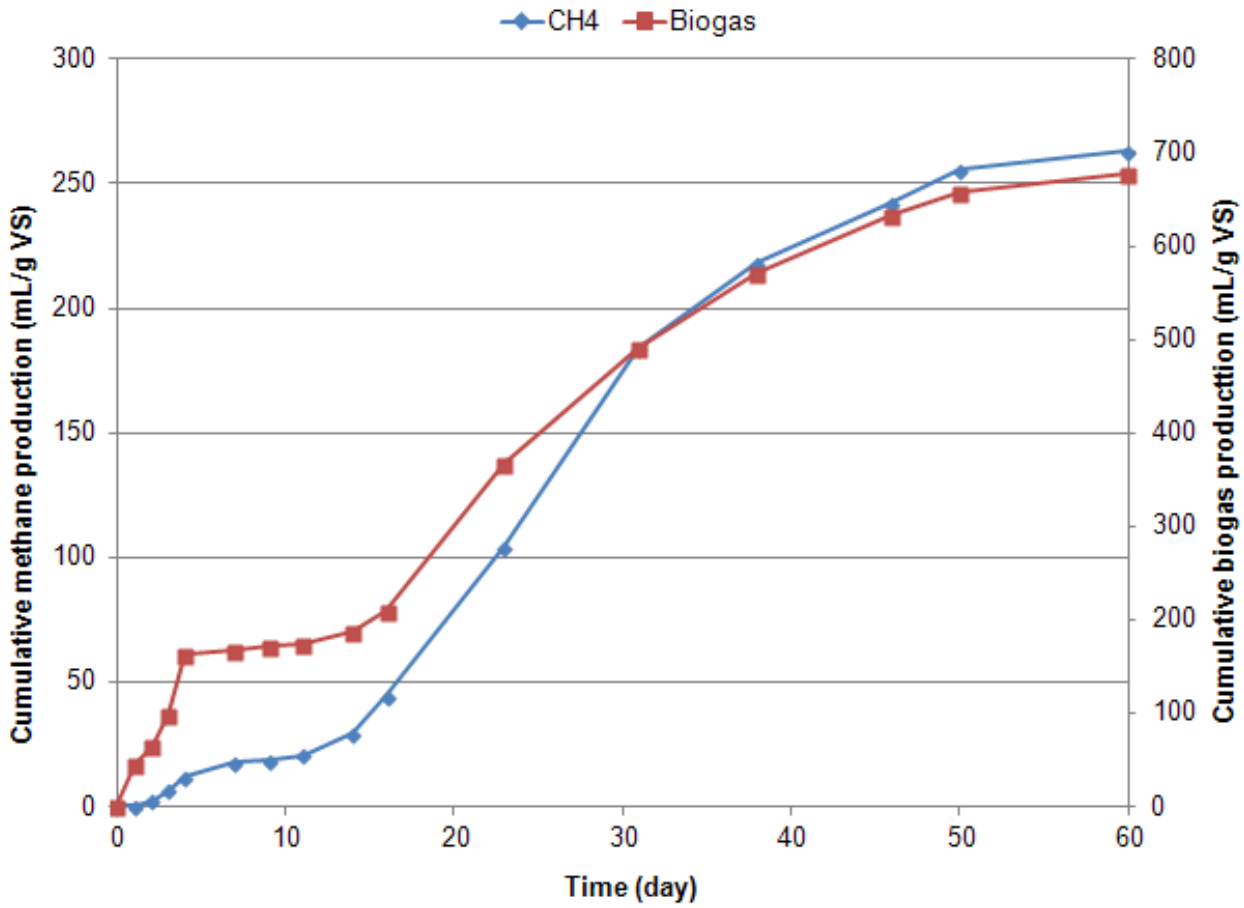

Figure 2. Cumulative biogas and methane production from the solid residues.

This integrated process is a sustainable alternative to incineration. The toxic ash produced in incineration process is useless and has to be stored in landfills. Moreover, the gases released to the environment causes global warming. Moreover, they contain nanoparticles such as dioxins, which is dangerous to human health. On the other hand, the remaining solid after this integrated process is non-toxic and non-carcinogenic to human health and can be used as soil conditioner. The main drawback of this integrated AD process is its long duration. In following studies, thermophilic AD process will be conducted. Moreover, the effect of different solid loadings will be studied to shorten the process time.

2. Plans for the Next 6 Months Period

- The effect of different solid loadings will be studied.
- Thermophilic AD process will be conducted.
- Final report and the 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				Year 3	
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
✓ 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										
Mesophilic AD of residues at different solid loadings										
Thermophilic AD of residues at different solid loadings										
Use of anaerobically digested solids as soil stabilizers										

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*	30	24
Numbers of publications in leading journals	2-3	5

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

We have 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, *Waste and Biomass Valorization* 5(6) 903-917.
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, *Biofuel Research Journal* (3) 98-105.
4. Uçkun Kiran, E., Trzcinski, A. P., Liu Y. 2014. Biorefineries for platform chemical production from food waste, *Journal of Chemical Technology and Biotechnology* DOI: 10.1002/jctb.4551.
5. Uçkun Kiran, E., Trzcinski, A. P., Liu Y. 2014. Enhancing the hydrolysis and methane production potential of mixed food wastes by a cost-effective enzymatic pretreatment, *Bioresource Technology* (Under revision).

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.

References

1. Gomez, X., Cuetos, M., Cara, J., Moran, A., Garcia, A. 2005. Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes: Conditions for mixing and evaluation of the organic loading rate. Renew. Energy, 31(12), 2017-2024.

2. Kim, J.H., Lee, J.C., Pak, D. 2011. Feasibility of producing ethanol from food waste. Waste Manag., 31, 2121–2125.

3. Cekmecelioglu, D., Uncu, O.N. 2013. Kinetic modeling of enzymatic hydrolysis of pretreated kitchen wastes for enhancing bioethanol production. Waste Manag., 33(3), 735-739.

4. Hong, Y.S., Yoon, H.H. 2011. Ethanol production from food residues. Biomass Bioenergy, 35(7), 3271-3275.

5. Jeong, S., Kim, Y., Lee, D. 2012. Ethanol production by co-fermentation of hexose and pentose from food wastes using Saccharomyces coreanus and Pichia stipitis. Korean J. Chem. Eng., 29(8), 1038-1043.

6. Moon, H.C., Song, I.S., Kim, J.C., Shirai, Y., Lee, D.H., Kim, J.K., Sung, O.C., Kim, D.H., Oh, K.K., Cho, Y.S. 2009. Enzymatic hydrolysis of food waste and ethanol fermentation. Internatl. J. Energy Res., 33(2), 164-172.

7. Pleissner, D., Kwan, T.H., Lin, C.S.K. 2014. Fungal hydrolysis in submerged fermentation for food waste treatment and fermentation feedstock preparation. Bioresour. Technol., 158, 48-54.

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.*