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Enhancing the hydrolysis and methane production potential of mixed food wastes by an effective enzymatic pretreatment

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Abstract

In this study, a fungal mash rich in hydrolytic enzymes was produced from the waste cake by solid state fermentation (SSF) of waste cake in a simple and efficient manner and was further applied for high-efficiency hydrolysis of mixed food wastes (FW). The enzymatic pretreatment of FW with this fungal mash resulted in 89.1 g/L glucose, 2.4 g/L free amino nitrogen and 165 g/L soluble chemical oxygen demand (SCOD) and 64% reduction in volatile solids within 24 hours. The biomethane yield and production rate from FW pretreated with the fungal mash were found to be respectively about 2.3 and 3.5-times higher than without pretreatment. After anaerobic digestion of pretreated FW, a volatile solids removal of 80.4±3.5% was achieved. The pretreatment of mixed FW with the fungal mash produced in this study is a promising option for enhancing anaerobic digestion of FW in terms of energy recovery and volume reduction.

Keywords:

Food waste; fungal mash; enzymatic pretreatment; solid state fermentation; anaerobic digestion; methane.

1 Introduction

Food waste (FW) is an organic waste originated from many various sources, e.g. households, cafeterias, restaurants etc. According to the Food and Agricultural Organization, one third of food produced for human consumption (i.e. nearly 1.3 billion tons) is lost or wasted throughout the food supply chain (FAO, 2012). Without proper treatment, one ton of FW can result in the emission of 4.5 tons of CO_2 in landfills (Kosseva, 2009). In Singapore, about 796,000 tons of FW was produced in 2013 (National-Environment-Agency, 2013) and the majority of which was incinerated with other combustible municipal wastes for volume reduction and recovery of heat and energy, while the residual ash was disposed of in landfill. It should be noted that incineration is not a preferable option of FW management the high moisture content of FW, high operation cost and generation of hazardous ashes and greenhouse gases (e.g. carbon dioxide)(El-Fadel et al., 1997). On the contrary, FW should be considered as a useful resource for producing high-value products (e.g. biofuels and platform chemicals) due to its organic-rich nature.

Anaerobic digestion of FW has been widely studied for biogas generation, which is a viable option for volume reduction of and energy recovery from FW (Uçkun Kiran et al., 2014). Although FW is readily biodegradable with a volatile solid fraction of up to 90%, the hydrolysis of solid FW into soluble organics has been known as the rate-limiting step of anaerobic digestion (Zhang et al., 2014). As a result, anaerobic digestion of FW has the drawbacks of long solid residence time and low conversion efficiency, indicating that a large anaerobic reactor is required (Quiroga et al., 2014). Therefore, different pretreatment methods of FW have been investigated for enhancing the hydrolysis of FW, e.g. ultrasonication (Li et al., 2013), microwave (Marin et al., 2010), thermochemical (Cavaleiro et al., 2013) and enzymatic hydrolysis (Moon & Song,

2011).Commercial enzymes including carbohydrases, such as glucoamylase, arabinase, cellulase, β-glucanase, hemicellulase, xylanase, proteases and lipases have been used to improve the hydrolysis of starch in FW (Moon & Song, 2011). The pretreatment of FW with multiple commercial enzymes appeared to be more efficient than that with a single commercial enzyme (Kim et al., 2006; Moon & Song, 2011). However, it should be realized that commercial enzymes are costly (e.g. about USD120 for treating one ton of FW with glucoamylase and alpha-amylase at 10 U/g FW) and generally available in single-type form. In order to make the enzymatic hydrolysis of FW more cost-effective, the enzymes should be produced *in situ* from a cheap feedstock without complex and costly downstream separation and purification steps.

So far, various kinds of FWs have been used to produce enzymes including proteases, cellulases, amylases, lipases and pectinases particularly through solid state fermentation (SSF). SSF has several advantages over submerged fermentation (SmF): (i); cost and energy-effective; (ii) a simple fermentation medium; (iii) superior productivity and (iv) less waste water generated. Higher enzyme yields can be obtained using SSF as it provides a similar environment to the microorganism's natural environment which provides better conditions for its growth and enzymes production (Thomas et al., 2013). Melikoglu (2008) developed a multi-enzyme solution of glucoamylase and protease during solid-state fermentation of waste bread using *A. awamori*. This solution was used for the hydrolysis of waste bread and wheat flour. Recently, this concept was also applied for the enzymatic hydrolysis of mixed food waste to produce a fermentation medium (Pleissner et al., 2014), which was further used as a nutrient-complete feedstock for the cultivation of microalgae (Lau et al., 2014; Pleissner et al., 2013) and succinic acid production (Sun et al., 2014). Therefore, this study aimed to (i) in-situ produce a fungal mash rich in

glucoamylase with FW as feedstock and (ii) investigate its application for the enzymatic pretreatment of FW with the focuses on enhancing the hydrolysis, biomethane production and waste volume reduction.

2 Materials and methods

2.1 Cake wastes for the production of fungal mash

In our previous work, bakery wastes, particularly waste cake, were found to be a good substrate for glucoamylase (GA) production (Uçkun Kiran et al., 2014). In this study, *Aspergillus awamori* obtained from ABM Chemicals Ltd (Cheshire, England) was used to produce GA with waste cake collected from a local catering as substrate through Solid State Fermentation (SSF). The waste cake was first ground, sieved and then stored in zipped plastic bags at -20^{o}C for further experiments. Mixed FW used in this study was collected from a cafeteria at Nanyang Technological University, and was homogenized by a blender immediately after the collection. The homogenized FW in zipped plastic bags were then stored at -20^{o}C for further use. The compositions of waste cake and mixed FW are presented in Table 1. For the purpose of comparison, commercial enzymes, e.g. α-amylase and glucoamylase from Genencor, Danisco Singapore Pte Ltd, were also employed in this study. The optimal pH ranges for α-amylase and glucoamylase were 5.0 - 5.8 and 4.2 - 4.8, respectively. It appears from Table 1 that proteins are not the main component of FW, thus commercial proteases were not tested in this study. Nevertheless, free amino nitrogen concentration in hydrolyzate was determined, which is an indication of the presence of proteases in the fungal mash.

Table 1. Composition of food wastes per gram of dry mass.

	Starch (mg)	Reducing sugar (mg)	Protein (mg)	Lipid (mg)	Ash (mg)
Waste cake	458±30	168±5	141±8	161±7.5	39± 2
Mixed FW	461±32	82±7	111±18	153±21	21±1

2.2 Production of fungal mash

Waste cakes with a particle size of 1.2 to 2.0 mm were used as sole carbon source for producing an enzyme cocktail using solid state fermentation in which moisture content was adjusted to 70% (wb) with 0.1 M phosphate buffer (pH 7.9). After sterilization by autoclaving at 120^{o}C for 20 min, the flasks were cooled down and then inoculated with *Aspergillus awamori* to obtain a spore concentration of 10^6/g substrate and the contents were mixed thoroughly with a sterile spatula. 10 g of such mixture was distributed into several identical Petri dishes and incubated at 30°C for 6 days under stationary conditions. The GA activity of the fungal mash harvested from two identical Petri dishes was found to be 113.7±5.2 U/gram dry solids. Fungal mash, i.e. the GA-rich fermentation solids were obtained at the end of the fermentation, and it was directly used to hydrolyze mixed FW without further separation of produced enzymes.

2.3 Hydrolysis of FW

Blended domestic food waste was inoculated with the fungal mash produced in this study at at a substrate loading of 50% (w/v) and a GA loading of 10 U/g dry FW. Hydrolysis was performed in duplicate in Duran bottles in a water bath shaker at 60^{o}C and 100 rpm for 24 h. For the purpose of comparison, similar experiments were also conducted in duplicate with commercial enzymes at 8.6 U/g dry FW for α-amylase and 10 U/g dry FW for GA. Samples taken at different time intervals were centrifuged at 10,000 rpm for 5 min before the analyses. The hydrolysis efficiency and solid mass reduction were determined by soluble COD and content of volatile

suspended solid after the pretreatments. Detailed experimental procedure is presented in Figure 1.

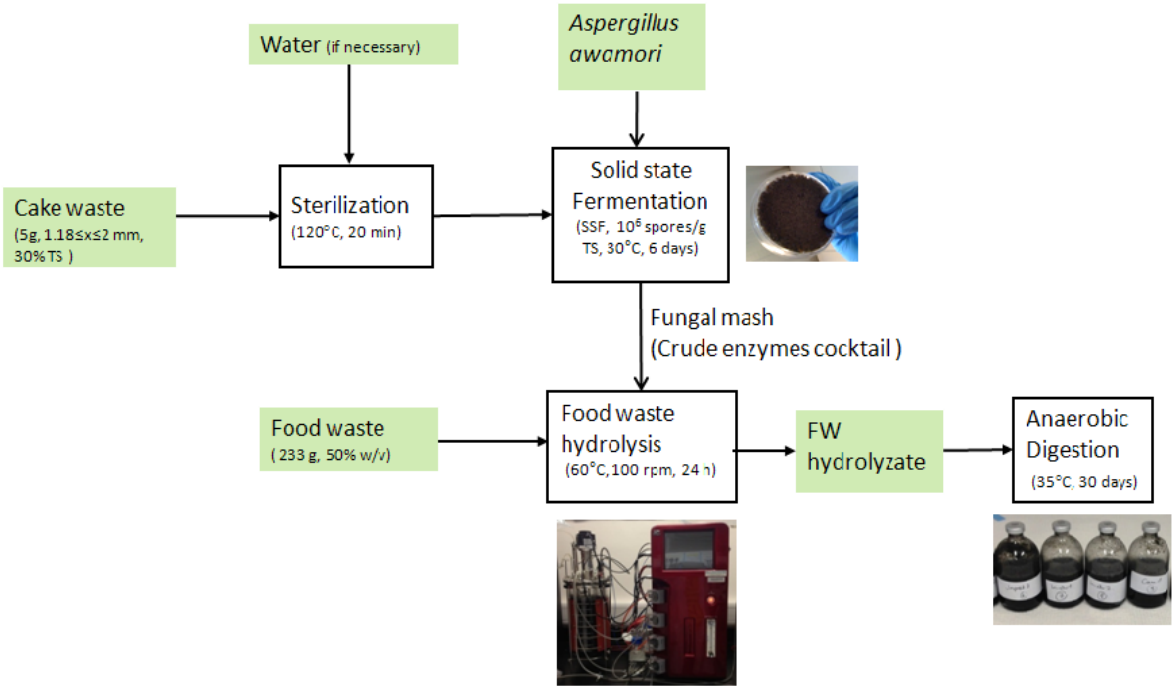


Figure 1. Experimental procedure.

2.4 Anaerobic digestion of enzymatically pretreated FW

The inoculum used for the anaerobic digestion was taken from a local full-scale anaerobic digester. After filling up the bottles with the respective amounts of pretreated FW (281 mg TS, 270 mg VS), 36.35 mL inoculum (32.3 g/L TSS, 14.87 g/L VSS) and anaerobic biomedium (30 mL), the headspace was purged with N_2 gas at 1 L/min for 3 min, and was then sealed immediately with rubber lids and metal caps to maintain anaerobic condition (Trzcinski and Stuckey (2012). Biochemical Methane Potential (BMP) of FW was determined in duplicate on an orbital shaker operated at 35^{o}C and150 rpm.

2.5 Analytical methods

Moisture and ash contents of FW were determined by analytical gravimetric methods (AOAC, 2001). Crude protein content was measured using HR Test'n tube TN kit (HACH, US) and calculated according to the Kjeldahl method with a conversion factor of 6.25. Starch content was determined using Megazyme's TN kit (Bray, Ireland). The lipid content was determined by hexane/isopropanol (3:2) method (Hara & Radin, 1978). The glucose concentration was determined with Optimum Xceed blood glucose monitor (Abbott Diabetes Care, Oxon, UK) (Bahcegul, 2011). Reducing sugars were quantified to monitor the saccharification of FW according to the dinitrosalicylic acid (DNSA) method using glucose as standard (Miller, 1959). Free amino nitrogen (FAN) concentration was measured in hydrolyzates using the ninhydrin reaction method (Lie, 1973). Soluble COD and volatile suspended solid reduction were determined using the standard methods (APHA-WPCF, 1998).

Protease activity was estimated through the formation of FAN by hydrolyzing 15 g/L casein solution (Sigma) at 60°C in 200 mM of citrate buffer at pH 4.8. One unit activity (U) was defined as the protease required for the production of 1 g FAN in 1 min. GA activity was determined with 2% (w/v) of soluble starch (Sigma) as substrate at 60°C and pH 4.8. One unit (1 U) of GA activity was defined as the amount of enzyme releasing 1 micromole glucose equivalent per minute under the assay conditions. All the analytical assays were conducted in triplicate.

The production yield and rate of biogas during anaerobic digestion of FW with and without pretreatment was evaluated by standard BMP tests. The contents of biogas were analyzed by gas

chromatography (Agilent 7890A) equipped with a thermal conductivity detector (TCD) and a HayeSep capillary column. The operational temperatures of the injector, detector, and column were set at 100, 150, and 115^{o}C, respectively. Helium at a flow rate of 35 mL/min was used as a carrier gas.

2.6 Data analysis

In this study, the modified Gompertz equation was used for comparing the kinetics of methane production from FW with and without pretreatment (Li et al., 2013):

$$B = B_0 \exp\left\{-\exp\left[\frac{R_m e}{B_0}(\lambda - t) + 1\right]\right\}$$

where B_0 is the estimated ultimate cumulative methane yield or methane production potential (mL/g VS), B is the cumulative methane yield (mL/g VS) at incubation time t (h), *e* is equal to 2.7183, R_m is the maximum methane production rate (mL/g VS h), and λ is the lag phase time (h).

3 Results and discussion

3.1 Production of glucose and free amino nitrogen from FW pretreated with fungal mash

Mixed food waste collected from a cafeteria at Nanyang Technological University was pretreated respectively with fungal mash produced in this study and commercial enzymes (e.g. alpha-amylase and glucoamylase).. It can be seen in Figure 2A that the highest glucose concentration of 89.1 ± 7 g/L was obtained after 24 hours in the experiment supplied with the fungal mash, while 77.2 ± 6.9 g/L was reached with the commercial enzymes. It should also be noted that the initial glucose production rate with the fungal mash was significantly higher than that with the

commercial enzymes. Given the complex composition of FW, different enzymes are required for high-efficiency hydrolysis and saccharification. Cekmecelioglu and Uncu (2013) developed a complex and costly pretreatment procedure using different kinds of enzymes namely as α-amylase, glucoamylase, cellulase and β-glucosidase. The highest glucose concentration achieved was only 64.8 g/L achieving 70% conversion after 6 hours of enzymatic hydrolysis of FW. This is lower than the glucose concentration of 76 g/L obtained in this study after 4 hours hydrolysis of mixed FW with fungal mash (Figure 2A). Although *A. awamori* is known to be an efficient producer of glucoamylases, it can also produce many different kinds of hydrolytic enzymes, such as amylases, proteases, cellulases and xylanases when growing on complex substrates, such as mixed FW in SSF (Koutinas et al., 2007; López et al., 2013). It had been reported that the fermented solids obtained from the SSF of babassu cake with *A. awamori* contained considerable activities of proteases, xylanases, and cellulase activities besides amylases (López et al., 2013). Table 2 shows that about 90-95% of starch in FW was hydrolyzed by the fungal mash produced in this study. This in turn suggests that this fungal mash contained some other carbohydrases such as α-glucosidases, β-amylases, β-glucanases pullulanases, cellulases, xylanases, hemicellulases, besides glucoamylase. Compared to commercial enzymes, the fungal mash produced in this study offers advantages over using extracted enzyme by reducing enzyme extraction step and thereby reducing economical constraint.

Table 2. Glucose, FAN and SCOD released from the hydrolysis of food waste after 24 h hydrolysis.

	Fungal mash		**Commercial enzymes**	
	Concentration (g/L)	**Conversion yield**	**Concentration (g/L)**	**Conversion yield**
Glucose	89.1 ± 7.0	90 - 95%[a]	77.2 ± 6.9	73 - 87%[a]

FAN	1.94 ± 0.12	72 - 80%[b]	0.10 ± 0.0	4%[b]
SCOD	164.7 ± 16.7	NA	128.5 ± 2.9	NA
VS reduction	NA	64%	NA	52%

NA: Not applicable, [a]: on starch basis (all the glucose produced was accounted for by the breakdown of starch), [b]: on protein basis.

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 conditions used in different studies using food wastes were very different from each other (Table 3). In the study by Pleissner et al. (2014), a glucose concentration of 143 g/L was obtained after 48 h fermentation at a FW loading of 43.2% (w/v) when solid mashes of *A. awamori* and *A. oryzae* were successively added at an interval of 24 h.

Table 3. Glucose concentrations and yields achieved using food waste.

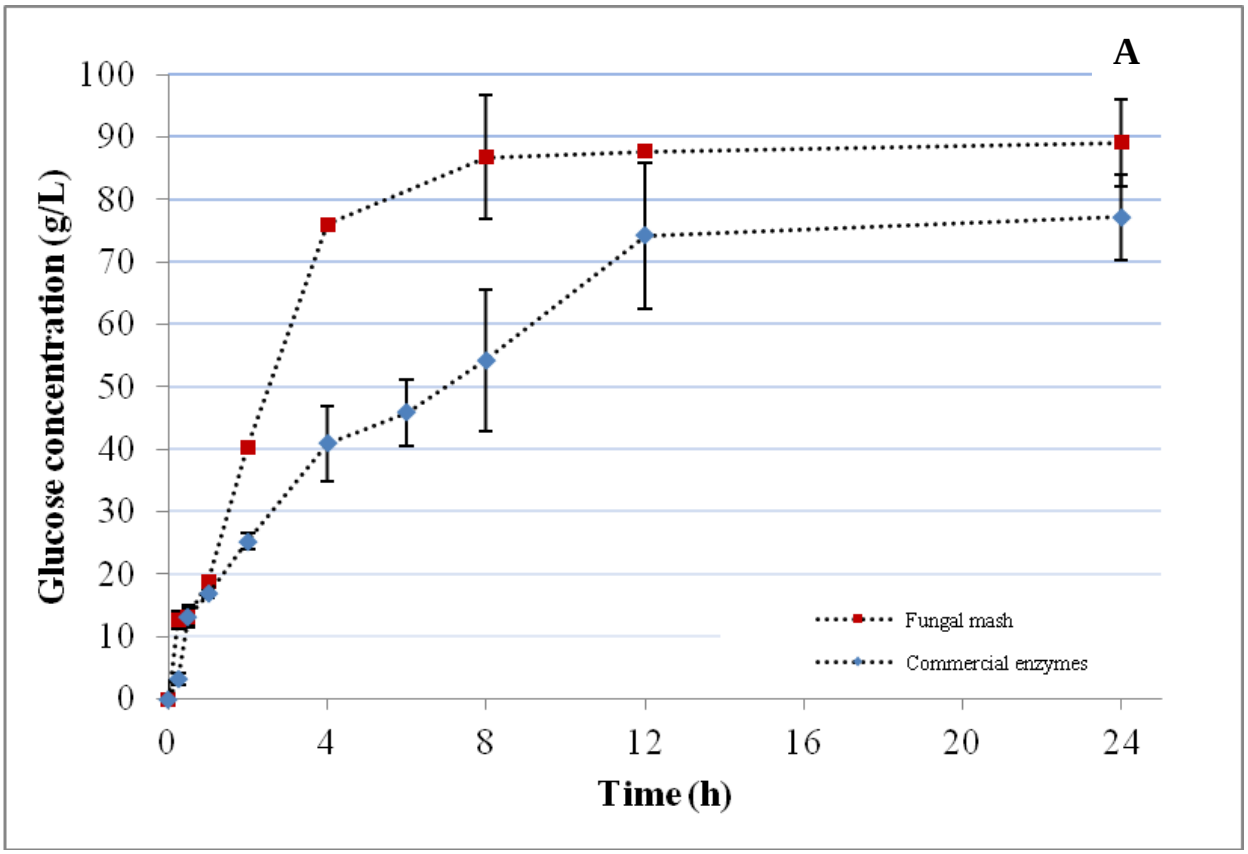
Enzymes	**$C_{Glucose}$** (g/L)	**Carbohydrate conversion rate** (%)	**Duration** (h)	**References**
GA, protease, cellulase	69.8	63[a]	12	Kim et al. (2011)
GA, cellulase, α-amylase, β-glucosidase	64.8	70	6	Cekmecelioglu and Uncu (2013)
GA, cellulase, α-amylase, β-glucanase, xylanase, hemicellulase, arabinase	79.1	NR	8	Jeong et al. (2012)
GA, cellulase, α-amylase, β-glucanase, xylanase, hemicellulase, arabinase	58.0	46[a]	6	Moon et al. (2009)
GA, α-amylase, β-glucosidase	65.0	NR	24	Hong and Yoon (2011)
GA, α-amylase, protease	119.2	66[a]	24	Sun et al. (2014)

Fungal mash (*A.awamori and A.oryzae*)	143.0	80-90[a]	48	Pleissner et al. (2014)
GA, α-amylase	74.3	78-82	12	This study*
Fungal mash (*A.awamori*)	87.7	85-95	12	This study*

GA: Glucoamylase, C: concentration, NR: not reported, *: substrate conversion rate (g glucose/g dry food waste).

The release of proteins during the hydrolysis of FW by the fungal mash was determined in terms of Free Amino Nitrogen (FAN) (Figure 2B). It was found that release of dissolved proteins was negligible during the pretreatment of FW with commercial enzymes as they did not contain any protease. In contrast, total FAN content in the hydrolyzates obtained during the pretreatment of FW with the fungal mash produced in this study quickly reached 1.94 g/L after 2 hours, and then stabilized at 2.4 g/L after 24 hours. This can be explained by the protease activity detected in the fungal mash (e.g.1.37±0.4 U/gds). The total nitrogen analysis revealed that 72 - 80% of proteins in FW were solubilized by the fungal mash. Moreover, proteases was able to help to hydrolyze carbohydrates by breaking down the bindings of proteins (Kim et al., 2006). Hence, the solubilization of FW was enhanced through the synergistic actions of the various kinds of enzymes present 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) ratios were 16.7 and 68.4 in the hydrolyzates obtained from the FW pretreatment with the fungal mash and commercial enzymes, respectively. It has been reported that a feedstock with a C/N ratio greater than 30 is considered deficient in nitrogen for a biological treatment process (Gomez et al., 2005;

Kayhanian & Rich, 1995). Therefore, the hydrolyzate obtained from the hydrolysis of FW with fungal mash is a good biomedium for subsequent biological processes, e.g. anaerobic digestion.



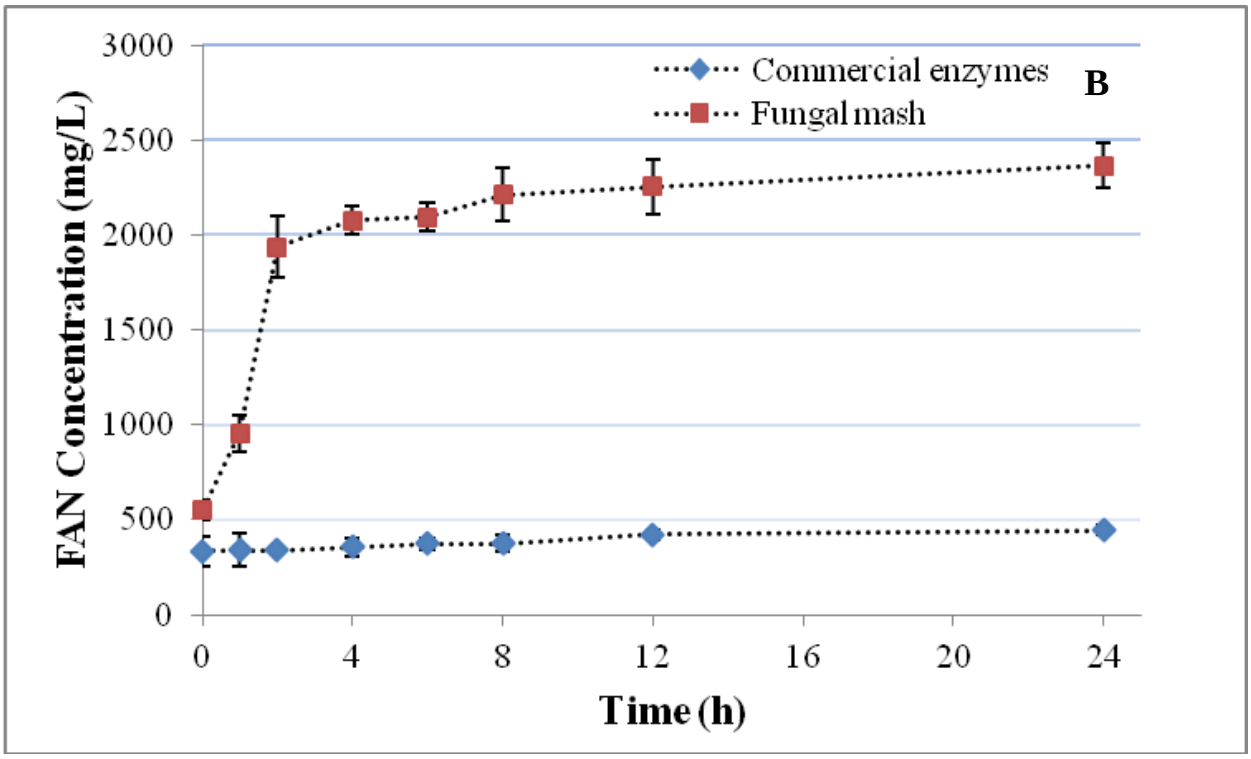


Figure 2. Effect of enzymatic pretreatment on glucose (A) and FAN (B) production from FW. Each data point is the average of triplicate measurements from duplicate experiments and the error bars represents the standard deviations.

3.2 Release of soluble COD in FW pretreatment with fungal mash

Figure 3 shows that the soluble COD (SCOD) concentration increased significantly in the first four hours of the pretreatment of FW with the fungal mash and commercial enzymes, respectively. The highest SCOD concentration of 164.7±16.7 g/L was obtained in the FW pretreatment with the fungal mash versus 128.5 ± 2.9 g/L for the commercial enzyme. Moreover, 64.3 ± 8.9% and 52 ± 4.9% reduction in volatile suspended solids were achieved at the end of the FW pretreatments with the fungal mash and commercial enzymes, respectively. This indicates

that the FW pretreatment using the fungal mash itself would lead to a volume reduction of more than 64% within 24 hours.

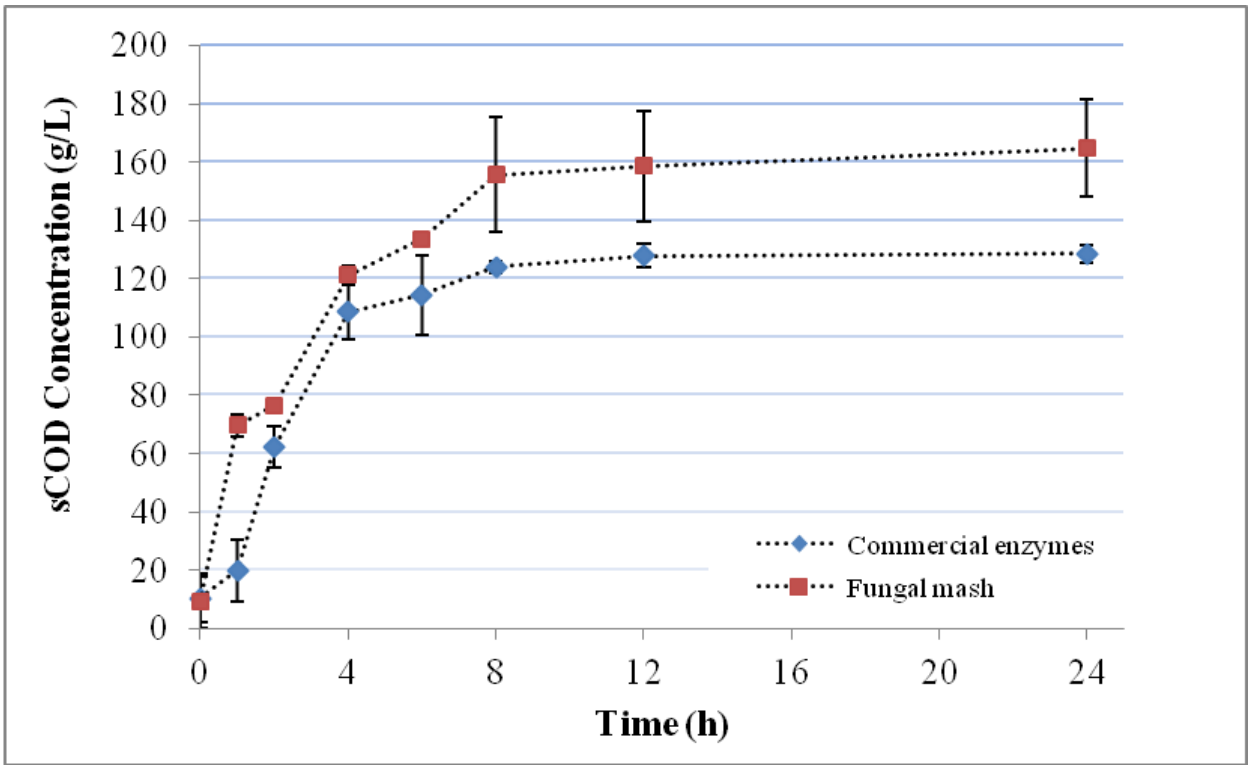


Figure 3. Effect of enzymatic pretreatment on soluble COD production from FW. Each data point is the average of triplicate measurements from duplicate experiments and the error bars represent the standard deviations.

3.3 Anaerobic digestion of FW pretreated with the fungal mash

To maximize volume reduction and energy recovery from FW, the pretreated FW subsequently underwent anaerobic digestion. Figure 4 shows the cumulative methane yield during the anaerobic digestion of pretreated FW with the fungal mash and commercial enzymes, respectively. The methane yields obtained in these two cases were found to be comparable.

However, the methane production from the fungal mash pretreated FW was faster than that of commercial enzymes pretreated FW.

The pretreatments provided almost a full conversion to biogas in the first 13 days. Later on, the bioconversion to biogas slowed down. Another advantage of the process is that an anaerobic digester with a short residence time (about 2 weeks) would be required, reducing the capital costs significantly in full scale application. Untreated FW had a lower gas production, indicating that a large fraction of biopolymers in FW without pretreatment would not be readily biodegradable. This shows the importance of proper pretreatment in the anaerobic digestion of FW. Fig. 4 indeed provides direct evidence that the fungal mash produced in this study can significantly enhance hydrolysis of FW, and improve the efficiency of subsequent anaerobic digestion. In addition to the biogas recovery, it should also be pointed out that 80.4 ± 3.5% of the overall reduction of volatile solids was achieved after anaerobic digestion of FW pretreated with the fungal mash. The integrated FW pretreatment-anaerobic digestion approach developed in this study appears to be a promising option for a better food waste management in terms of energy recovery and volume reduction.

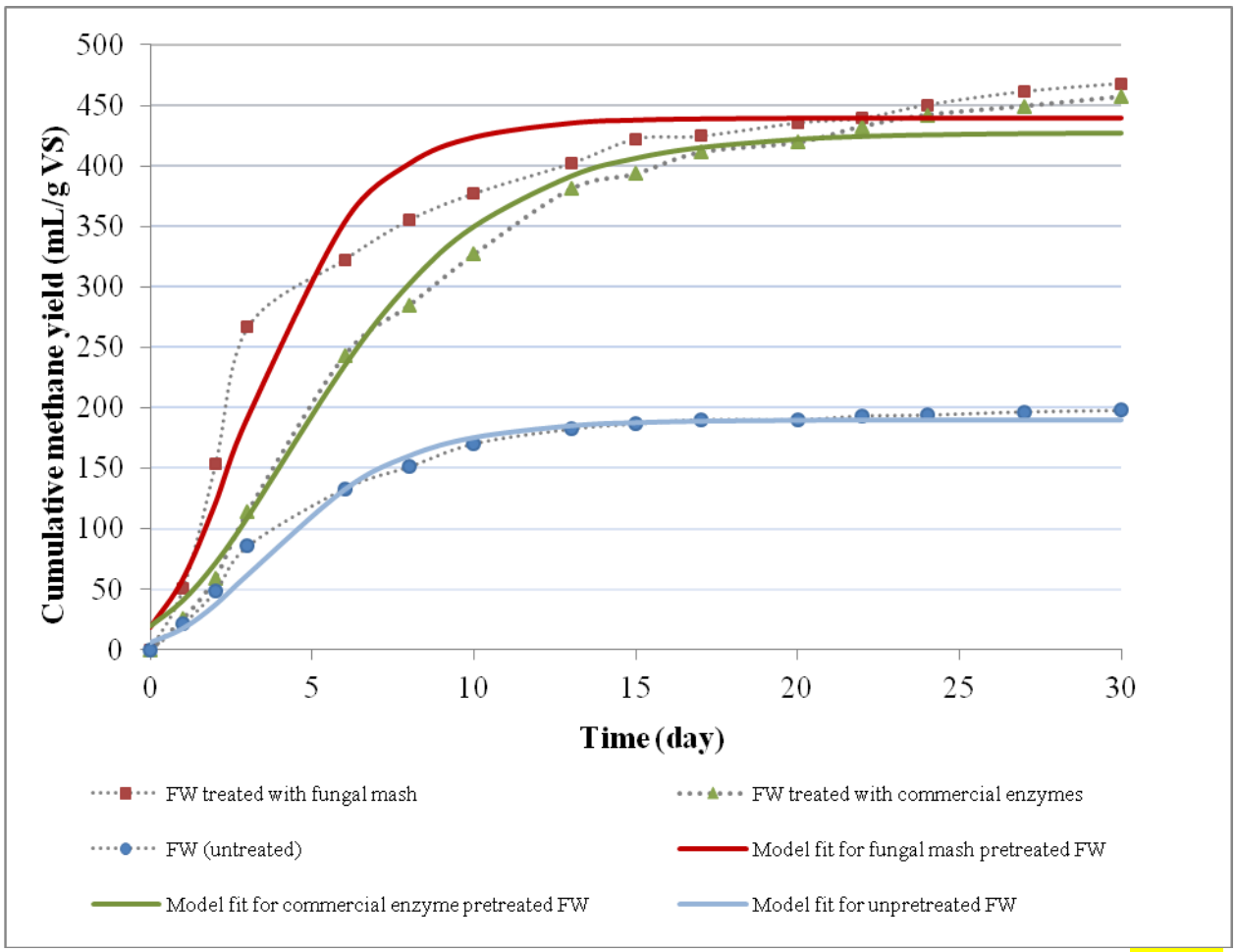


Figure 4. Effect of enzymatic pretreatment on cumulative methane production from FW. Results are the average of two replicates. Error was within ± 5 mL/g VS. Solid lines indicate the the simulation of the experimental data using the Gompertz Equation.

The experimental data presented in Figure 4 were fitted into the Gompertz equation, and the constants estimated are summarized in Table 3. A shorter lag phase of 8 hours was observed in the anaerobic digestion of FW pretreated with the fungal mash, whereas 12 hours for commercial enzyme and 16 hours for untreated FW. The highest methane production rate was obtained in the case where FW was pretreated with the fungal mash. For example, the anaerobic digestion of

FW pretreated with the fungal mash was about 1.9 -times and 3.5 -times faster than the pretreatment with commercial enzymes and untreated FW, respectively.

Table 3. The parameters estimated for the anaerobic digestion of FW pretreated with different methods.

	FW without pretreatment	FW pretreated with commercial enzymes	FW pretreated with fungal mash
λ (hours)	16	12	8
R_m (mL CH_4/g VS. h)	1.1	2.0	3.8
B_0 (mL CH_4/g VS)	190	428	440
Experimental yield (mL CH_4/g VS)	197.9	457.3	468.2

λ: lag phase time, R*m:* maximum methane production rate, B_0:estimated ultimate cumulative methane yield.

4. Conclusions

A fungal mash rich in glucoamylase and protease was produced from cake wasteand was applied for enzymatic hydrolysis of mixed FW. The enzymatic pretreatment using this fungal mash was shown to be more efficient than commercial enzymes. The biomethane yield and production rate from FW pretreated with the fungal mash were found to be respectively 2.3- and 3.5-times higher than without pretreatment. The overall volatile suspended solid destruction in the process was 80.4±3.5%. These results showed that direct use of the fungal mash produced in-situ enzymes purification steps is a promising option for food waste treatment.

Acknowledgements

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