



5<sup>th</sup> International Conference of the International  
Commission of Agricultural and Biosystems Engineering  
(CIGR)

Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB)  
Virtually from Québec City, Canada – May 11-14, 2021



**FOOD WASTE ANAEROBIC CO-DIGESTION WITH CRUDE GLYCEROL: IMPROVE  
METHANE YIELD BY COMBINED PRETREATMENTS OF HYDROTHERMAL PROCESSING  
AND LIPASE ADDITION**

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**CSBE21812 – Presented at SPECIAL SESSION on biogas**

**ABSTRACT** To enhance anaerobic biodegradability, pretreatments and co-digestion with other biomass wastes could be effective methods to eliminate the hydrolysis rate limitation for achieving high performance of food waste (FW) anaerobic digestion. In this study, the use of lipase addition (LA, 0.5g/L), hydrothermal pretreatment (HTP, 120°C for 60 min) and a combination of both methods (HTP + LA) were applied to hydrolyze the organic matters in FW, and also the effects of crude glycerol (CG) as co-substrate was assessed with 0%, 5% and 10% of volatile solids (VS).

Batch tests showed that untreated FW co-digestion with CG resulted in higher methane yields. In particular, the maximum specific methane yield (SMY) potential of 214.3NL CH<sub>4</sub>/kg VS occurred at a substrate ratio of 90:10 (FW:CG, %VS), compared to mono-digestion. However, for pretreated FW, the CG addition exerted a negative impact on anaerobic digestion performance as the proportion of CG increased because of propionic acid accumulation acidifying the reactors thus breaking the balance of the anaerobic system and inhibiting the growth of methanogens. Among these three types of pretreatments, it was demonstrated that the pretreatment of FW by HTP combined with LA advanced the appearance time of methane peaks and would be a promising option for enhancing SMY value (642.8NL CH<sub>4</sub>/kg VS) as well as for maximizing methane potential according to the modified Gompertz model.

**Keywords:** Food waste; Lipase addition; Hydrothermal process; Anaerobic digestion; Biogas

**INTRODUCTION** Nowadays, approximately 1.3 billion tons of food wastes were generated annually across the entire food supply chain and the global food waste accounted for 8-10% of all man-made greenhouse gas emissions and also caused an annual loss of US\$1 trillion (Mbow et al., 2017). The traditional practices like incineration,

composting and landfill could not meet the demand of sustainable development and even cause other negative effects on the environment. Developing more appropriate measures for tackling food waste is emerging as one of the key issues. Anaerobic digestion is widely accepted as suitable technology to treat food waste to produce biogas while reducing the secondary pollution of the environment during the digestion process(Li et al., 2013).

Crude glycerol is the main by-product of biodiesel industry through the transesterification of vegetable oil, animal fat or used kitchen oil with alcohol, which accounts for about 10% of the initial feedstock weight and has a lot of impurities like ethanol, water, salt, heavy metals, free fatty acids, unreacted monoglycerides, diglycerides, triglycerides, and methyl esters(S.Robra et al., 2010). The interests of mixing food waste and crude glycerol have been stimulated because (1) the high water content in the food waste can act as a solvent for crude glycerol; (2) glycerol provides easily biodegradable material.

From the aspects of food waste characteristics, the crude lipid and crude protein contents are in the ranges of 22.8–31.45% and 14.71–28.64% respectively. The order of the hydrolysis rate of food waste compositions was given as lipids < proteins < carbohydrates(Christ O et al., 2000). This implies that lipids hydrolysis is the limiting-step for the whole anaerobic process of food waste. In addition, the high lipid content in food waste could possibly result in lipid dross accumulation in the digester, and pose adverse effects such as the use of organic matters by methanogens and the equipment cleanings. Under these circumstances, a high-efficiency, mild reaction condition, no secondary pollution pretreatment technology is extremely needed.

In this study, lipase as a kind of efficient catalyst was chosen to treat food waste to hydrolyze the oil slick in food waste. At the same time, hydrothermal pretreatment (HTP) which considered as a thermal treatment method which promotes the dissolution of recalcitrant organic compounds by decomposing cell membranes efficiently at an appropriate temperature and residence time(Tekin et al., 2014)also were put into practice to effectively shorten the hydrolysis time of food waste, change the physical and chemical properties of proteins, carbohydrates and lipids inside and improve the subsequent biological treatment efficiency.

**MATERIALS AND METHODS** The anaerobic sludge used as the inoculum was obtained from Hokkaido NO.1 farm and domesticated at 52°C. The raw materials used as substrate were food waste collected from the central restaurant of HOKKAIDO University and crude glycerol derived from transesterification process during biodiesel production from Revo International Company. The food waste was minced and homogenized by using a blender and then screened. The sample was stored at -20°C before use.

The lipase used in this study was purchased from AMANO ENZYME INC (Head office, Japan). The source of the lipase is *Pseudomonas fluorescens*. The application conditions

of lipase is pH 7-8.5, temperature 50-60°C . Accurately weigh 25 mg lipase and dissolved in 50 mL Sodium Chloride solution (10g/L) cooling to <10°C .

To obtain appropriate application conditions for Lipase, firstly adjust the pH of food waste to 7.5 with 4g/L sodium carbonate solution and add 50% w.w. enzyme solution to the processed food waste then incubate at 52°C for 10 hours, no longer adjust the pH in the subsequent process.

The hydrothermal pretreatment of FW was carried out in autoclave (TYPE: PPY-CHA, TOKYO RIKAKIKAI CO, LTD) with volumes of 50 mL. The reactor was operated at 120°C for 60 min. The time was measured from when the autoclave reached the set temperature. The reactor was cooled to ambient temperature after the pre-treatment.

Combined pretreatments was carried out by above-methioned steps. Add lipase solution to hydrothermal treated food waste.The anaerobic digestion condition was keeping the organic load rate (OLR) is equal to 1 g-VS/kg-inoculum/day, 52°C for 7 days.

## RESULTS AND DISCUSSIONS

**Anaerobic performances** The anaerobic biodegradability was investigated from the specific biogas yield (SBY) and specific methane yield (SMY). From the perspective of SBY and SMY, for FW without any pretreatment, co-digestion with CG can increase methane production and the ratio of FW : CG (VS provided) = 90:10 (CG10 group)is better. The possible reason may be the superior anaerobic biodegradability of CG which had an important effect on parameters related to the matter content such as VS/TS and C/N. Under the feeding situation of raw FW, the addition of CG (10% VS provided) led to an increase in the VS/TS ratio from 88.83% to 90.65% and bring about an increase of 178.75% methane accumulation. These results indicated that the methane production are substantially affected by substrate availability and characteristics of itself, which might contribute to meet the requirements of microorganisms to achieve optimal microbial activities.

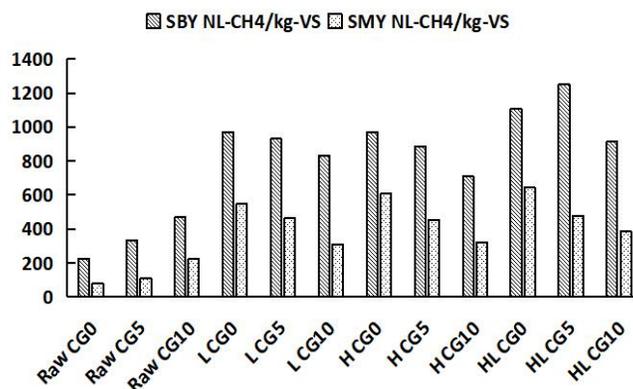


Figure 1. SBY and SMY of different experimental groups (raw FW without any pretreatments, lipase addition, hydrothermal processing and combination of these two methods; CG0: no CG, CG5: 5% CG provided, CG10: 10% CG provided).

$$SBY[L - CH_4 / g - VS] = \frac{\text{Biogas accumulation [L - CH}_4\text{]}}{\text{Amount of volatile solids input [g]}} \quad (1)$$

$$SMY[L - CH_4 / g - VS] = \frac{\text{Methane accumulation [L - CH}_4\text{]}}{\text{Amount of volatile solids input [g]}} \quad (2)$$

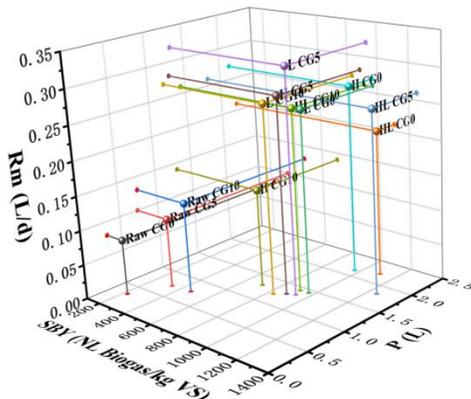
Pretreatments proposed significant effects on the improvement of anaerobic digestion performance of FW. Single digestions of FW treated by lipase hydrolysis, hydrothermal and combination of the two methods without CG had the SMY value of 546.9, 606.5, 642.8 NL CH<sub>4</sub> (kg·VS)<sup>-1</sup> (normal liters of methane per kg of volatile solids), respectively. However, the SMY value of the three pre-treatment groups gradually decreased with the increase of the CG ratio, the possible reason may be the overloading of CG which contains various impurities.

$$H = P * \exp \left\{ - \exp \left[ \frac{R_m e}{P} (\lambda - t) \right] + 1 \right\} \quad (3)$$

Where H is the cumulative methane production (L) recorded at time t (d); P is the methane potential (L); R<sub>m</sub> is the maximum methane production rate (L/d); e is exp (1) = 2.718; and λ is the lag-phase period (h).

The modified Gompertz model (Elbeshbishy & Nakhla, 2012), shown in Eq.(3) was used for curve fitting of the values of biogas and methane production. The kinetic constants for anaerobic digestion under the different treatment conditions were determined then the dynamic process was simulated and the methane production potential of the fermentation substrate was quantitatively analyzed. The fitting of this model and the 3D plots were made using Origin 2020b software (OriginLab Corporation, Northampton, MA, USA). The data obtained from all the experimental groups were checked for goodness of fit with the model and evaluated using values of Pearson correlations coefficients (SPSS Statistics, IBM, Armonk, NJ, USA).

(a)



(b)

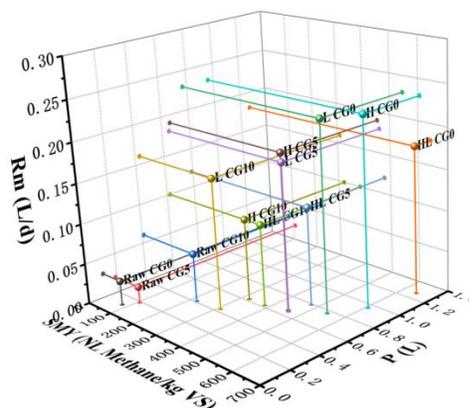


Figure 2. 3D scatter plot of P, Rm (predicted from Gompertz model) against (a) SBY and (b) SMY.

3D scatter plot shown in Figure 2 indicated the variations of P, Rm, and SBY or SMY value under different treating methods and the SBY, SMY from experimental data were practically in accordance with the predicted P and Rm value from Gompertz model. Overall, the combined pretreatment for lipase addition and hydrothermal process had obvious promotion on the digestion performance. Each kilogram of FW treated with combined methods had higher methane potential. The Pearson correlations between the relationships of SBY and P, SBY and Rm, SMY and P, SMY and Rm were 0.895, 0.828, 0.954 and 0.892, respectively. Correlation was significant at the 0.01 level (2-tailed).

**CONCLUSION** Co-digestion with 10% (VS provided, v/v) CG showed the best results in terms of the raw FW biodegradability enhancement. The pretreatments can significantly increase the production rate and the total volume of methane. Especially, the combination method of hydrothermal and lipase addition receives the highest SMY potential ( $642.8 \text{ NL CH}_4 (\text{kg VS})^{-1}$ ) and  $\text{CH}_4/\text{CO}_2$  ratio (1.679). However, utilizing CG to replace parts of total volatile solids of FW presents suppression phenomenon with the gradual increasing proportion of CG due to the propionic acid accumulation.

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