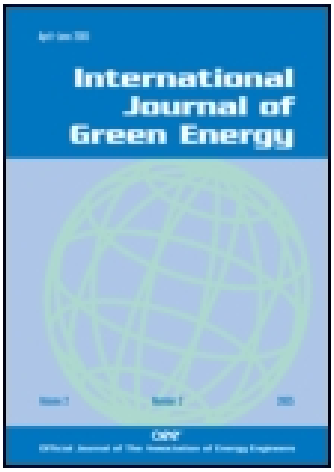

International Journal of Green Energy
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ljge20

Enzymatic Hydrolysis of FoodWaste and Methane Production Using UASB Bioreactor

Hee Cheon Moon [a] & I. S. Song [a]
[a] Department of Environmental Research , Gyeonggi-do Institute of Health and Environment , Suwon, Korea
Published online: 25 Apr 2011.

To cite this article: Hee Cheon Moon & I. S. Song (2011) Enzymatic Hydrolysis of FoodWaste and Methane Production Using UASB Bioreactor, International Journal of Green Energy, 8:3, 361-371, DOI: 10.1080/15435075.2011.557845

To link to this article: http://dx.doi.org/10.1080/15435075.2011.557845

International Journal of Green Energy, 8: 361–371, 2011

ISSN: 1543-5075 print / 1543-5083 online
DOI: 10.1080/15435075.2011.557845

ENZYMATIC HYDROLYSIS OF FOOD WASTE AND METHANE PRODUCTION USING UASB BIOREACTOR

Hee Cheon Moon and I. S. Song

Department of Environmental Research, Gyeonggi-do Institute of Health and Environment, Suwon, Korea

Complex carbohydrates of food waste (FW) can be converted to biogas, methane. In this article, enzymatic solubilization of FW and methane production potential using an upflow anaerobic sludge blanket (UASB) reactor of FW liquor enzymatically hydrolyzed were investigated. The optimum conditions of FW hydrolysis were enzyme mixture ratio of 1:2:1 with carbohydrase: protease: lipase, respectively, 0.2% (w/w FW) of mixture dose, and 10-h hydrolysis reaction. More than 95% of high soluble chemical oxygen demand (SCOD) removal efficiency and 0.35 L-CH_4/g-SCOD of high methane yield were observed at 9.1 g-SCOD/L/d of organic loading rate. Our results revealed that methane production via a UASB reactor in conjunction with enzymatic hydrolysis of FW could be a novel anaerobic digestion process to obtain high-value biogas.

Keywords: ***Food waste; Enzymatic hydrolysis; Methane fermentation; UASB bioreactor***

INTRODUCTION

Food waste (FW) discharged from households, restaurants, and food industry accounted for 23% of municipal solid wastes in Korea; approximately 11,237 tons of FW per day were generated in 2003 (Ministry of Environment 2008). FW has been recognized as pollutants, since it readily decomposes, generates odors, and sometimes causes illness under natural condition, due to its high biodegradable organic compound and moisture content. On the other hand, FW comprises a rich source of carbon and nutrients, e.g., soluble sugar, starch, lipid, protein, cellulose, and other inorganic materials, for microbial growth; for this reason, it has been utilized as an alternative nutrient medium for propionic acid (Moon et al. 2005), lactic acid (Praneetrattananon et al. 2005a), succinic acid (Praneetrattananon et al. 2005b), ethanol (Moon et al. 2009), hydrogen and methane fermentation (Han and Shin 2004a). Especially, the application of its carbon substrate in methane fermentation has the considerable potential to reduce environmental problems associated with the disposal of FW and to produce methane, which can be a promising alternative to fossil fuel.

Although FW was considered readily biodegradable since the volatile fraction was as high as 90% of total solids (TSs), its degradation of particulates into soluble substrates was still a rate-limiting step; as the result, the anaerobic digestion of FW required a long time

Address correspondence to Hee Cheon Moon, Department of Environmental Research, Gyeonggi-do Institute of Health and Environment, Suwon, 440-290, Korea. E-mail: hichun@gg.go.kr

(10 d) using currently available methods (Kim, J. K., et al. 2006). The hydrolysis rate of the substrate, which is defined as the degradation rate of organic compounds in substrate particulates, is an important factor in determining the hydraulic retention time (HRT) of FW in methane production. Also, differences in individual biodegradability and the hydrolysis rate of complex FW compositions, e.g., starch, cellulose, protein, and lipid, generates various degradation characteristics that may cause decreases in fermentation efficiency, by means of an accumulation of volatile fatty acids (VFA), a decrease in pH, and product inhibition in anaerobic digestion (Han and Shin 2004b). To preclude such problems and enhance the hydrolysis rate of volatile solids (VSs) in FW, there were several studies into various pre-treatments, including those that were steam explosion (Nakamura and Sawada 2003), thermochemical liquidization (Shigeki et al. 1997), and enzymatic hydrolysis (Kim, H. J., et al. 2006).

Among the high-rate anaerobic reactors, upflow anaerobic sludge blanket (UASB) reactor has been widely used to treat various kinds of industrial wastewater. In UASB reactor, anaerobic bacteria are immobilized by a process whereby bacteria are spontaneously aggregated, resulting in dense granules featuring a high level of microbial activity and good settling characteristics (Lettinga et al. 1980). The great success of UASB reactor lied in their ability to retain a high concentration of granular sludge, which allowed for high organic loading rate (OLR) and the maintenance of long retention time for biological solid.

This article investigated the effect of enzymatic hydrolysis on the solubilization of volatile suspended solid (VSS) in FW by means of commercial enzymes such as carbohydrase, protease, and lipase. The feasibility of methane production in conjunction with UASB bioreactor was evaluated using the liquid phase of FW hydrolyzed by three-enzyme combination.

MATERIALS AND METHODS

Composition of FW

The FW used in this experiment was collected from a cafeteria in the Gyeonggi Small and Medium Business Center (Suwon, Korea). Bones and shells were removed from the FW. Table 1 illustrated the characteristics of the FW in detail; the percentages of vegetable, grain, and meat matter in the wet FW were 49%, 31%, and 17% (w/w), respectively. It was crushed in a homogenizer (AM-11; Tokyo, Japan) and sieved through a wire mesh (2×2 mm). The pH was 4.3 ± 0.1, owing to a considerable amount of pickled-food residues and the generation of VFA during storage and transportation. Also, it had $81.5 \pm 1.3\%$ (w/w) of moisture content, $18.5 \pm 0.3\%$ of TS matter, and $95.7 \pm 0.5\%$ of VS in TS. VSS, total chemical oxygen demand (TCOD), and soluble chemical oxygen demand (SCOD) were 91.0 ± 3.6 g/L, 263.5 ± 12.5 g/L, and 127.4 ± 5.2 g/L, respectively.

Enzymatic Hydrolysis of FW

Three types of commercial enzymes were used in the enzymatic hydrolysis of FW. Their characteristics were listed in Table 2. Carbohydrase, a multi-enzyme complex containing arabinase, cellulase, β-glucanase, hemicellulase, and xylanase, extracted from *Aspergillus. Aculeatus* was purchased from Novozyme. (Novozyme Korea, Seoul, Korea). Protease, a fungal protease/peptidase complex, originated from *Aspergillus oryzae* and a

Table 1 Characteristics of food waste used in this study.

Item	Unit	Value
pH	–	4.3 ± 0.1
Moisture content	% (w/w)	81.5 ± 1.3
TS	% (w/w)	18.5 ± 0.3
VS	% TS (w/w)	95.7 ± 0.5
Ash	% TS (w/w)	4.1 ± 0.2
VSS	g/L	91.0 ± 3.6
TSCOD	g/L	263.5 ± 12.5
SCOD	g/L	127.4 ± 5.2
Composition		
Vegetable	% (w/w)	49.0
Grain	% (w/w)	31.0
Meat	% (w/w)	17.0

Data were mean values ± standard deviation of three replicates.

Table 2 Characteristics of commercial enzymes.

Enzyme	Carbohydrase	Protease	Lipase
Origin	*Aspergillus aculeatus*	*Aspergillus oryzae*	*Candida rugosa*
State	Liquid	Powder	Granular
Product name	Viscozyme® L	Protease M "Amano"	Lipase AY "Amano" 30
Application	Brewing industry Fruit water treatment Cell-wall degrading enzyme	Protein hydrolysis (Meat/bone stocks)	Fatty acid hydrolysis

triacylglycerol lipase derived from *Candida rugosa* were purchased from Amano Enzyme Inc. (Nagoya, Japan).

Five hundred grams of minced FW and 500 g of distilled water were mixed in a 2-L Erlenmeyer flask. The pH was adjusted to 4.5 using 3N NaOH. This mixture was used as a sample for all enzymatic hydrolyses of FW; all enzymatic reactions were conducted in a shaking incubator (DSK 512, Seoul, Korea) at 50°C and 150 rpm for 24 h. First, the single enzyme hydrolysis of FW using carbohydrase, protease, and lipase was carried out at different levels with each enzyme from 0.02 to 0.4% (w/w FW). Second, four levels of mixed enzyme ratio were tested with the 0.4% of mixed-enzyme dosage; 1: 1: 1, 2: 1: 1, 1: 2: 1, and 1: 1: 2 of carbohydrase: protease: lipase (C: P: L), respectively, to determine the optimal enzyme mixture ratio. Third, based on the results of the preliminary experiments, a mixed-enzyme ratio of 1: 2: 1 with C: P: L, which shown the highest VSS degradation, was employed to determine the optimal dosage of mixed enzyme from 0.02 to 0.4% (w/w FW). Sample was taken at regular time intervals and the VSS and SCOD contents were monitored during the enzymatic hydrolysis of FW.

Methane Fermentation Using UASB Bioreactor

Based on the experiments, the 1: 2: 1 mixed-enzyme ratio of C: P: L, 0.2% of the mixed-enzyme dosage, and 10 h of reaction time were selected as the optimum condition for the enzymatic hydrolysis of FW. FW hydrolysate was centrifuged at 4,000 rpm for

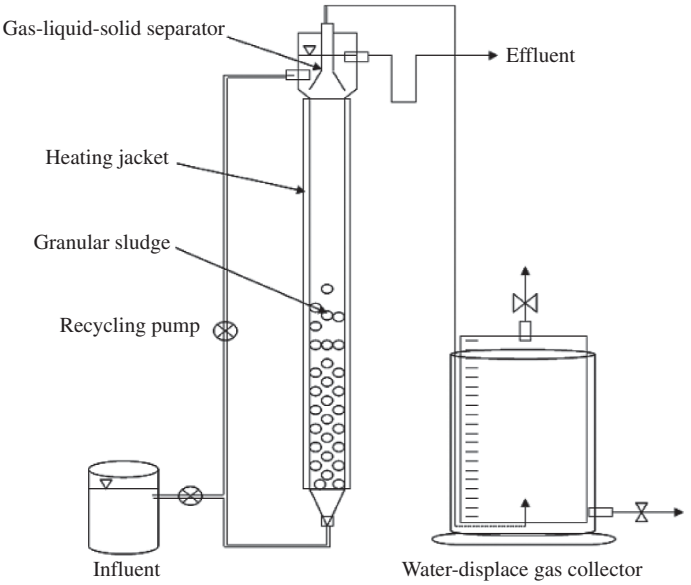

Figure 1 Schematic diagram of UASB bioreactor.

10 min and the supernatant was diluted five times with tap water; it was then used as an influent for a UASB bioreactor. The UASB reactor consisted of a main body [6 cm (internal diameter) × 66 cm (height)], gas-liquid-solid separator [10 cm (internal diameter) × 15 cm (height)], and 2.7 L of working volume, as illustrated in Figure 1. The inoculum, 25% (v/v) of working volume, was granular sludge taken from the full-scale UASB reactor that processed brewery wastewater. The granular sludge was acclimated with a diluted liquid phase of FW hydrolysate for 1 month. The superficial flow velocity (υ_s) of the reactor was maintained at 1.0 m/h by controlling the recycling flow rate of 92 L/d with a peristaltic pump. By using a heating jacket, the operational temperature of the reactor was maintained at mesophilic temperature (35 ± 2°C). The OLR was raised stepwise from 1.6 to 13.2 g-SCOD/L/d by increasing influent flow rate during a 75-day operation. Incremental change in each OLR was made when the coefficient of variation in the biogas generation was less than 10% (Hill and Bolte 2000). The reactor was completely sealed through the use of packing rubber. Daily biogas production was monitored using a 20 L of water-displacement gas collector.

Analysis

Moisture, TS, VS, VSS, and ash content were analyzed in accordance with standard methods (Greenberg, Clesceri, and Eaton 1992). SCOD after filtration (Whatman GF/C) and TCOD without filtration were measured by the sealed tube method (sample size = 2 mL) using DR/2000U spectrophotometer (Hach, Loveland, CO, USA). The CH_4 content in the biogas was daily analyzed with a gas chromatograph (Hewlett-Packard 6890 series; IL, USA) equipped with a flame ionization detector (FID) and a DB-TPH capillary column (30 m × 0.32 mm × 0.25 μm). The operational temperatures of the injector, detector, and

column were kept at 100, 250, and 50°C, respectively. Helium was used as a carrier gas, at a flow rate of 40 mL/min.

RESULTS AND DISCUSSION

Single Enzyme Hydrolysis of FW

FW consisted of easily biodegradable organic solids, i.e. carbohydrate, protein, and lipid from grains, vegetables, and meat. Therefore, effectively converting organic polymers into liquor, which could be readily metabolized by acidogens/methanogens, was crucial for achieving high methane production from FW. Six levels of individual-enzyme dosage including control were added to each sample; control (no added enzyme), 0.02%, 0.05%, 0.1%, 0.2%, and 0.4% (w/w FW). VSS reduction rate was employed as a principle parameter for evaluating the efficiency of enzymatic hydrolysis of FW. In the control sample, in which no enzyme was added, a slight VSS reduction, approximately 8%, was observed after 24-h shaking incubation. It seemed to be the result of the activity of micro-organisms that were naturally contaminated in FW and/or partial hydrolysis by the NaOH added for adjusting the pH to 4.5. VSS reduction rate was hyperbolically raised in accordance with increase in the level of each enzyme as illustrated in Figure 2. Up to 0.1% (w/w FW) dosage of lipase and carbohydrase, net VSS reduction rate significantly increased and reached 11% and 29%, respectively. Beyond 0.1% of enzyme dosage, a distinctive increase of VSS reduction rate was not shown. In fact, lipase was very efficient in hydrolyzing oil compounds in FW; a non-oil band in hydrolysate was observed after hydrolysis, but it was not effective in reducing VSS. In case of protease, the greatest efficiency of net VSS

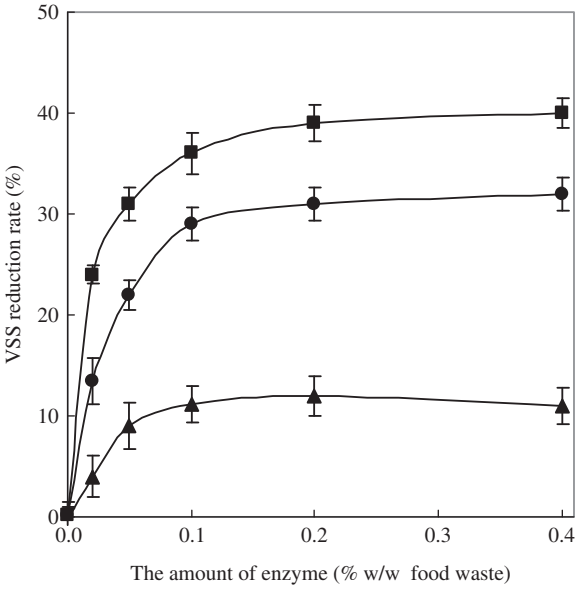

Figure 2 VSS reduction rate by single enzyme hydrolysis of food waste. All experiments were conducted at 50°C at pH 4.5 for 24 h. The bar represents standard deviation ($n = 3$). Legend: (■ = protease, ● = carbohydrase, ▲ = lipase).

reduction, approximately 39%, was achieved at 0.2% of protein dosage. A slight increase in VSS reduction was observed at over 0.2%. Among the three types of enzyme, the most significant VSS reduction was obtained by addition of protease even though FW contained high carbohydrate content. Protease may be more efficient than carbohydrase for solubilization of complex carbohydrate in FW. It has been proposed that the biopolymer of some complex carbohydrates was combined by lectin-like proteins binding polysaccharides that were cross-linked to adjoining proteins (Higgins and Novak 1997). It was also reported that protease was the most dominant enzyme for FW liquation in its application for alternative organic carbon source of biological nutrient removal system (Kim, H. J., et al. 2006). Protease could release carbohydrate by breakdown of binding proteins. This fact may explain the role of protease in the FW degradation. Release of readily soluble polysaccharides through protease activity can enhance FW solubilization.

Mixed Enzyme Hydrolysis of FW

Various enzyme combinations were investigated using an equivalent dosage, 0.4% (w/w FW), to evaluate the effect of mixed-enzyme ratio on FW solubilization. 49 ± 0.5%, 54 ± 0.4%, 61 ± 0.3%, and 51 ± 0.5% of VSS reduction rate were observed at 1: 1: 1, 2: 1: 1, 1: 2: 1, and 1: 1: 2 of C: P: L ratio, respectively, as illustrated in Table 3. VSS reduction efficiencies of all mixed-enzyme ratios were higher than those of the single enzyme hydrolysis. As expected from the result of the single-enzyme hydrolysis, the highest VSS reduction rate was achieved at a 1: 2: 1 of C: P: L ratio, which contained a higher proportion of protease. Therefore, the 1: 2: 1 ratio of C: P: L was chosen for a performance to determine the optimal dosage of enzyme mixture. The different amounts of mixed enzyme were added: control (no added enzymes), 0.02%, 0.05%, 0.10%, 0.20%, 0.30%, and 0.40% (w/w FW). VSS and SCOD concentration were monitored during 24-h hydrolysis period, as shown in Figure 3a and b, respectively. As mentioned, approximately 8% VSS reduction and 11% SCOD production in control sample were also observed after 24-h incubation period. Almost all FW solubilization occurred in early stage, within 10-h incubation and after 10 h of enzymatic reaction, nearly constant VSS and SCOD content were obtained. Up to 0.2% dosage, the degree of VSS reduction and the corresponding SCOD generation were markedly greater in accordance with increase of mixed-enzyme dosage. However, beyond 0.2% dosage, the increase of both parameters was not noticeable. At 0.2–0.4% of mixed-enzyme dosage, the lowest VSS and the highest SCOD content were obtained: approximately 17,000 and 102,000 mg/L, which corresponded to 61% VSS reduction and 62% SCOD generation, respectively. Our result was comparable to the outcome reported by

Table 3 Effect of mixed enzyme ratio on VSS reduction rate of food waste.

Mixing ratio of enzymes (C: P: L)[1]	VSS reduction rate (%, w/w)
1: 1: 1	49 ± 0.5
2: 1: 1	54 ± 0.4
1: 2: 1	61 ± 0.3
1: 1: 2	51 ± 0.5

[1]Mixed-enzyme ratio of carbohydrase, protease and lipase. All performances were run with 0.4% (w/w FW) of mixed-enzyme dose at pH 4.5, 50°C for 24-h shacking incubation. Data were mean values ± standard deviation of three replicates.

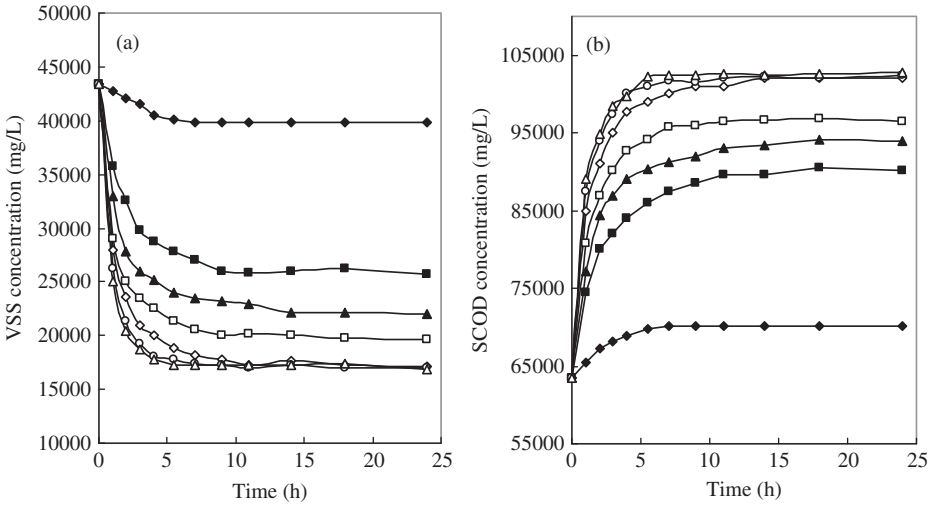

Figure 3 VSS (a) and SCOD (b) concentration in accordance with time course at various dosages of mixed-enzyme ratio with 1: 2: 1 of carbohydrase: protease: lipase. Legend: (□ = control, ■ = 0.02%, ▲ = 0.05%, □ = 1.0%, ◇ = 0.2%, ○ = 0.3%, □ = 0.4%).

Kim, H. J., et al. (2006), who achieved a 52.7% of net VSS reduction from the enzymatic solubilization of FW by commercial enzyme mixture. Parmer, Singh, and Ward (2001) observed that the addition of a cocktail of protease, lipase, and hemicellulase to anaerobically digested sludge resulted in a 29% reduction in TSS, over a 96-h period. Roman, Burgess, and Pletschke (2006) also found that the addition of a commercially available enzyme combination containing cellulase and protease to sewage sludge resulted in a 60% net TSS reduction during a 5-day digestion period. The results presented here demonstrated that enzymatic hydrolysis could effectively release associated organic material entrapped in FW particulate, as well as sewage sludge, and accelerated their solubilization, ultimately enhancing the utilization of organic matter as a substrate for anaerobic bacteria.

The correlation between net VSS reduction and SCOD production was estimated with all data of Figure 3a and b. A ratio of 1.29 g-SCOD/g VSS and high coefficient of determination ($r^2 = 0.98$) between both parameters were obtained, as plotted in Figure 4. This result indicated that a 1.29 g-SCOD would be produced when 1 g VSS in FW was hydrolyzed by enzyme combination.

Methane Fermentation of FW Liquor Using UASB Bioreactor

FW hydrolysate solubilized by optimum hydrolysis condition; 0.2% (w/w FW) of mixed-enzyme dosage, a 1: 2: 1 ratio of C: P: L, and 10-h incubation period, was centrifuged and the supernatant was diluted five times. The diluted liquor was used as influent of UASB reactor. The influent contained 20.5 ± 0.3 g/L of SCOD and its pH was 4.5 ± 0.2. It was continuously fed into the reactor with stepwise increase in SCOD loading rate from 1.6 to 13.2 g SCOD/L/d. Figure 5 showed the temporal variation of pH (a), methane production rate (b), SCOD removal efficiency (c), biogas and CH_4 yield (d), and SCOD

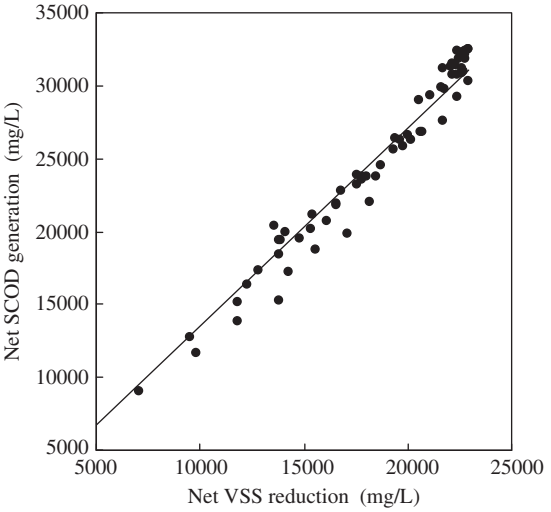

Figure 4 Net SCOD generation and VSS reduction by a mixed enzyme hydrolysis of food waste.

loading rate (e) during the operation of the UASB reactor. Although the influent with a pH 4.5 ± 0.2 was introduced to the reactor without any pH adjustment, the pH of the effluent was kept within a range of 8.3–7.6 up to a loading rate of 9.1 g SCOD/L/d (Figure 5a). This result meant that the buffer capacity of liquor hydrolyzed from FW was sufficient in maintaining the stable performance of the UASB reactor. The CH_4 production rate gradually increased in accordance with a shift in SCOD loading rate: 0.49, 1.04, 1.54, and 2.31 L-CH_4/L-reactor/d at 1.6, 3.4, 4.7 and 7.1 g-SCOD/L/d of OLR, respectively, as shown as Figure 5(b). The maximum methane production of 3.1 L-CH_4/L-reactor/d was obtained at 9.1 g-SCOD/L/d. The high SCOD removal efficiency of 98–99% was able to be observed at 1.6–7.1 g-SCOD/L/d of OLR. Thereafter, as shown in Figure 5(c), a slight decrease of SCOD removal efficiency, 95%, was observed at 9.1 g-SCOD/L/d of OLR. The organic compounds in FW liquid hydrolyzed by mixed enzyme were removed by the UASB reactor with high treatment efficiency at a short HRT of 2.2 d. These results indicated that enzymatic solubilization of FW could be beneficial as a pre-treatment for anaerobic digestion and the UASB method could be useful for rapid and high efficient digestion of FW liquor. However, a continuous increase in the OLR to 11.3 and 13.2 g-SCOD/L/d resulted in a significant drop in all parameters of anaerobic digestion: to 2.5 and 2.0 L-CH_4/L-reactor/d of methane production rate, 7.2 and 6.6 of pH, and 77% and 66% of SCOD removal efficiency, respectively, due to excessive SCOD loading. Figure 5d showed the change in daily biogas production. The biogas yield gradually increased as the SCOD loading rate increased; the average biogas production reached 0.42–0.44 and 0.48 L-gas/g-SCOD at loading rates of 1.6–7.1 and 9.1 g-SCOD/L/d, respectively. However, at the range of SCOD overloading, 11.3–13 g-SCOD/L/d, biogas yield significantly decreased to 0.39–0.46 L-gas/g-SCOD. Methane yield was accompanied by increase in SCOD loading rate. The highest methane yield of 0.35 L-CH_4/g-SCOD was obtained at 9.1 g SCOD/L/d, which was higher than that of FW digestion by thermophilic three-stage methane fermentation (0.22 L-CH_4/g-SCOD) (Kim, J. K., et al. 2006) and comparable to that of FW liquor by thermochemical liquidization (0.30–0.40 L-CH_4/g-SCOD) (Tsukahara et al. 1999). High methane content of 67–75% was obtained at a SCOD loading rate of 1.6–9.1 g-SCOD/L/d. However, SCOD overloading reduced methane content to 53–68%.

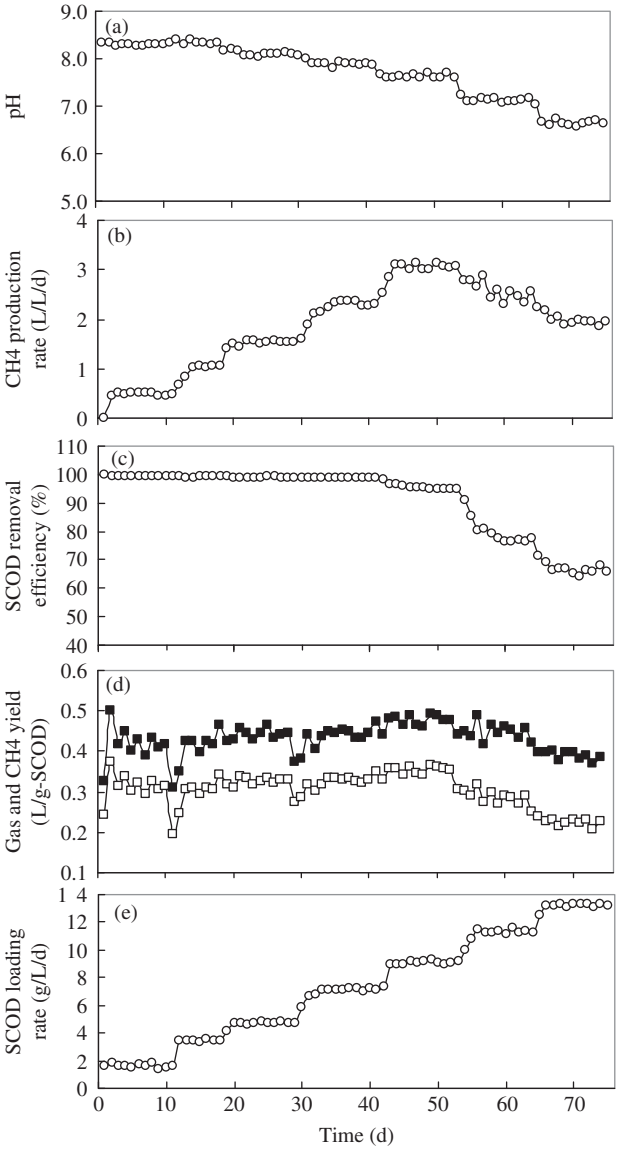

Figure 5 Evolution of different parameters in the performance of UASB reactor: pH (a); CH_4 production rate (b); SCOD removal efficiency (c); gas and CH_4 yield (d); SCOD loading rate (e). Legend: (■ = gas yield, □ = CH_4 yield).

CONCLUSION

Effect of enzymatic hydrolysis of FW on VSS reduction and SCOD generation was experimentally investigated. Mesophilic methane fermentation of the FW liquid phase hydrolyzed by enzyme combination was conducted using a UASB reactor. The following findings were obtained:

The experiment on FW hydrolysis by individual enzyme revealed that protease was the most dominant enzyme among three types of enzymes for liquidizing VSS. The condition performed by using a 1: 2: 1 ratio of C: P: L, a mixing dosage of 0.2% (w/w FW), and an 10-h reaction time was found to be the most appropriate for FW hydrolysis in view of providing VSS reduction (61%) and SCOD generation (62%). The ratio of net SCOD generation to net VSS reduction was 1.29 g-SCOD/g-VSS during the mixed-enzyme hydrolysis of FW. While running the UASB reactor employing enzymatically hydrolyzed FW liquor as influent, consistent SCOD removal efficiency (more than 95%), high methane content (67–75%), and high methane yield (0.35 L-CH_4/g-SCOD) was observed at the loading rate of 9.1 g-SCOD/L/d, which was equivalent to HRT of 2.2 d. In conclusion, our results mean that the combination of enzymatic hydrolysis of FW and methane fermentation using UASB reactor can be a promising methane production process which efficiently decreases HRT by increasing the hydrolysis rate of organic solid, resulting in enhancing anaerobic digestion of FW. Moreover, the environmental benefits of employing FW as an alternative energy resource for methane production could be a significant driving force encouraging expanded use of biomass for energy production. The fundamental environmental reasons for employing FW, as methane production, will be a global greenhouse gas control and local or regional solid waste and water quality control.

REFERENCES

Greenberg, A.C., L.S. Clesceri, and A.D. Eaton. 1992. *Standard methods for the examination of water and wastewater*, 18th ed. American Public Health Association (APHA).

Han, S.K., and H.S. Shin. 2004a. Biohydrogen production by anaerobic fermentation of food waste. *International Journal of Hydrogen Energy* 29: 569–77.

Han, S.K., and H.S. Shin. 2004b. Performance of an innovative two-stage process converting food waste to hydrogen and methane. *Journal of the Air & Waste Management Association* 54: 242–49.

Higgins, M.J., and J.T. Novak. 1997. Characterization of exocellular protein and its role in bioflocculation. *Journal of Environmental Engineering* 127: 479–85.

Hill, D.T., and J.P. Bolte. 2000. Methane production from low solid concentration liquid of swine waste using conventional anaerobic fermentation. *Bioresource Technology* 74: 241–47.

Kim, H.J., Y.G. Choi, G.D. Kim, and T.H. Chung. 2006. Effect of enzymatic pretreatment on acid fermentation of food waste. *Journal of Chemical Technology & Biotechnolgy* 81: 974–80.

Kim, J.K., B.R. Oh, Y.N. Chun, and S.W. Kim. 2006. Effects of temperature and hydraulic retention time on anaerobic digestion of food waste. *Journal of Bioscience and Bioengineering* 102, no. 4: 328–32.

Lettinga, G., S.W. vanVelson, W. Hobma, W. deZeeuw, and A. Klapwyk. 1980. Use of the upflow sludge blanket reactor concept for biological waste water treatment anaerobic treatment. *Biotechnology and Bioengineering* 22: 699–734.

Ministry of Environment, Republic of Korea. 2008. Internet homepage of the Ministry of environment. http://www.me.go.kr.

Moon, H.C., I.S. Song, C.J. Kim, Y. Shirai, D.H. Lee, J.K. Kim, S.O. Chung, D.H. Kim, K.K. Oh, and Y.S. Cho. 2009. Enzymatic hydrolysis of food waste and ethanol fermentation. *International Journal of Energy Research* 33: 164–72.

Moon, H.C., M. Wakisaka, Y. Shirai, and M. Taniguchi. 2005. Preferential substrate utilization by *Propionibacterium shermanii* in kitchen refuse medium. *Japan Journal of Food Engineering* 6: 37–43.

Nakamura, Y., and T. Sawada. 2003. Ethanol production from artificial domestic household waste solubilized by steam explosion. *Biotechnology and Bioprocess Engineering* 8: 205–9.

Parmer, N., A. Singh, and O.P. Ward. 2001. Enzyme treatment to reduce solids and improve settling of sewage sludge. *Journal of Industrial Microbiology* 26: 383–6.

Praneetrattananon, S., H.C. Moon, M. Wakisaka, and Y. Shirai. 2005a. Kitchen refuse utilization for succinic acid production by *Actinobacillus succinogenes* ATCC 55618. *Japan Journal of Food Engineering* 6: 259–67.

Praneetrattananon, S., M. Wakisaka, Y. Shirai, and V. Kitpreechavanich. 2005b. Kitchen refuse: A novel substrate for L (+)-lactic acid production by *Rhizopus oryzae* in submerged fermentation. *Japan Journal of Food Engineering* 6: 45–51.

Roman, H.J., J.E. Burgess, and B.I. Pletschke. 2006. Enzyme treatment to decrease solids and improve digestion of primary sewage sludge. *African Journal of Biotechnology* 5: 963–7.

Shigeki, S., T.M. Seiichiinoue, T. Kenichiro, and O. Tomoko. 1997. Thermochemical liquidization and anaerobic treatment of kitchen garbage. *Journal of Fermentation and Bioengineering* 83: 451–5.

Tsukahara K., T. Yagishita, T. Ogi, and S. Sawayama. 1999. Treatment of liquid fraction separated from liquidized food waste in an upflow anaerobic sludge blanket reactor. *Journal of Bioscience and Bioengineering* 87: 554–6.