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Elucidating a synergistic effect of food waste addition on the enhanced anaerobic digestion of waste activated sludge

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Abstract−Waste activated sludge (WAS) has often been anaerobically digested with other types of organic waste, expecting a synergistic enhancement. In the present work, a slight amount of food waste (FW) such that the change of the substrate concentration and the C/N ratio could be neglected was added to WAS and biological methane potential tests were performed. As the amount of FW input increased, the total amount of CH_4 produced and CH_4 yield increased. The calculation proved that at least 30% of the increased amount of CH_4 produced was derived from WAS, clearly signalling a synergistic enhancement. Measurements of the hydrolytic extracellular protease activity and ammonia concentration support the finding of synergism in that the addition of easily biodegradable organics to WAS facilitated the degradation of protein, a major constituent of WAS. This is the first report clearly revealing a synergistic effect of FW addition on the enhanced digestion of WAS.

Keywords: Anaerobic Digestion, Biogas, Food Waste, Synergistic Effect, Waste Activated Sludge

INTRODUCTION

The increasing number of wastewater treatment plants (WWTPs) has resulted in the improvement of river water quality while also raising concerns over sludge management. In this regard, anaerobic digestion (AD) is considered a promising technology that can produce clean energy in the form of methane (CH_4) along with a reduction of the sludge volume [1]. There are two streams of sludge produced from WWTP: primary sludge and waste activated sludge (WAS). Primary sludge, which mainly consists of settleable organic matter collected from the first clarifier, is known to be more biodegradable than WAS [2]. The major fraction of WAS consists of microbial cells, and the cell walls of these cells offer some resistance to biodegradation. To enhance AD efficiency, various disintegration methods, such as ultrasonication, ozonation, acid/alkaline treatments, and microwave irradiation, have been applied to rupture these cells and hence gain readily biodegradable intracellular organics [3,4].

Another strategy to enhance the digestion efficiency of WAS is co-digestion with other forms of organic wastes [5-8]. The anaerobic co-digestion of different types of waste sources is a well-accepted means of improving digestibility and biogas production through synergistic and complementary effects, which offset the lack of nutrients and dilute harmful substances. The addition of food waste (FW) to WAS has often improved the performance, as FW has a higher organic content and good biodegradability as well as a lower ammonia level than WAS [7,9,10]. However, to the best of our knowledge, the synergistic biogas increase has not been clearly revealed yet. More precisely, although the total amount of biogas produced is increased via an addition of FW, it remains unknown as to whether the increased amount stems from FW or WAS. If synergism had occurred, a certain amount of increased biogas production should have come from WAS.

Hydrolysis is known as a rate-limiting step in the AD of WAS, and it can be mediated by the activity of hydrolytic extracellular enzymes (HEE) [11]. The well-known types of HEE in AD are protease, amylase, and lipase, all of which have specific functions with regard to degrading proteins, carbohydrates, and lipids, respectively [12,13]. They are induced at different secretion levels by anaerobes depending on the constitution of the organic polymers [13]. Given that WAS is mainly composed of protein, the level of hydrolytic extracellular protease (HEP) activity can be linked to the increased degree of hydrolysis and thus to enhanced AD efficiency. In addition, an increased ammonia level has often been observed with the

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disintegration of WAS [14,15].

This study aims to clearly reveal the synergistic effect of FW addition on the enhanced AD efficiency of WAS. A slight amount of FW such that the change of the substrate concentration and C/N ratio could be neglected was added to WAS and biological methane potential (BMP) tests were performed. Through a simple calculation, the existence of a synergistic effect was clarified. In addition, to reinforce the findings, the HEP activity and ammonia concentration were measured during AD of WAS and a mixture of WAS and FW.

MATERIALS AND METHODS

1. Preparation of Feedstock and Inoculum

The WAS and inoculum used here were taken from a gravity sludge thickener line and an anaerobic digester, respectively, at a local wastewater treatment plant. FW collected from a school (KAIST) cafeteria was shredded to a particle size of less than 5 mm by a grinder. The FW was then diluted with tap water to be 28 g/L on a chemical oxygen demand (COD) basis. The characteristics of the feedstock and inoculum used are listed in Table 1.

2. Experiment

Anaerobic batch tests were performed using a 250 mL serum bottle with an effective volume of 150 mL. After 45 mL of inoculum was placed into bottles, 87 mL of WAS equivalent to 20 g COD/L was added to all of the bottles. Then, a slight amount of diluted FW (28 g COD/L), ranging from 0 to 12 mL, was added in each case. Detailed information pertaining to the experimental design and the corresponding substrate concentration, nitrogen concentration, and C/N (COD/nitrogen) ratio are provided in Table 2. The remainder of the effective volume was filled with tap water, and the pH was adjusted at 7.5±0.1 by adding 6 N of KOH solution. The bottles were then purged with N_2 gas for 5 min to provide an anaerobic condition. Fermentation was carried out in a shaking incubator (100 rpm) held at a temperature of 35 °C. The tests were carried out in triplicate and the results were averaged.

Table 1. Characteristics of feedstock and inoculum

Item	Unit	Waste activated sludge	Food waste	Inoculum
TCOD	g COD/L	34.5	167	12
SCOD	g COD/L	0.3	67	2
TS	g/L	41	204	20
VS	g/L	24	136	10
Carbohydrate	g COD/L	4	89	1
Protein	g COD/L	22	39	7
Total nitrogen	mg N/L	2,360	5,180	850
Ammonia	mg NH_4^+-N/L	80	280	365
pH	-	7.4	4.3	7.3
Alkalinity	mg/L as $CaCO_3$	4,230	360	2,120
C/N ratio	g COD/g N	14.6	32.3	14.1

3. Analytical Methods

The contents of CH_4 and carbon dioxide in the biogas were analyzed by a GC (Gow-Mac series 580) equipped with a thermal conductivity detector (TCD) with a 1.8 m×3.2 mm stainless-steel column packed with Porapak Q (80/100 mesh) using helium as a carrier gas. The COD, pH and ammonia were determined according to standard methods [16]. The concentrations of carbohydrates and proteins were determined by a colorimetric method [17,18].

To calculate the CH_4 production, the mass balance was evaluated using headspace measurements of the gas composition and the total volume of biogas produced at each time interval [19]. The cumulative CH_4 production curve was described by the following modified Gompertz equation [20].

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

where M(t)=cumulative CH_4 production (mL) at cultivation time t (d); P=ultimate CH_4 production (mL); R_m=gas production rate (mL/d); λ=lag period (d); and e=exp(1)=2.71828.

The ammonia released during the AD of WAS is described as the first-order reaction [14].

$$\frac{dP'}{dt} = -kP' \quad (2)$$

$$P' = P'_0 + \alpha(1 - e^{-kt}) \quad (3)$$

where P'_0=initial ammonia concentration (mg/L); P′=ammonia concentration (mg/L); and k=first-order rate coefficient; and α= conversion coefficient, respectively.

4. Analysis of the HEP Activity

The HEP activity levels of the supernatants of batch tests 1 and 5, taken from each respective reactor, were tested. After centrifugation at 8,000 rpm for 15 min, all of the samples were immediately filtered through a 0.45 μm membrane filter (Whatman, USA) of cellulose acetate, which exhibits a very low protein binding capacity. Once the HEP extraction process was complete, the activity levels were directly tested by measuring the enzymatic release of trichloroacetic acid (TCA)-soluble peptides from azocasein (KFDA, 2004). The substrate solution contains 0.5% azocasein in 20 mM

Table 2. Experimental design and biological methane production performance

Added amount (mL)		Substrate conc. (g COD/L)	Total nitrogen (g N/L)	C/N ratio (g COD/g N)	CH_4 yield (mL CH_4/g COD_{added})	CH_4 production rate (mL CH_4/L/d)
Waste activated sludge	Food waste					
87	0	20.0	1.37	14.6	90±10	167±9
87	2	20.4	1.38	14.8	99±13	220±10
87	4	20.8	1.39	14.9	111±3	233±5
87	8	21.5	1.41	15.2	120±5	273±7
87	12	22.2	1.44	15.5	139±8	327±7

of Tris-HCl buffer (pH 8). A refrigerated 10% (w/v) TCA solution was used to stop the reaction. Reactions between 0.4 mL supernatant of the sample and the 1.6 mL of the substrate solution were incubated for 1 h at 35 °C. After 1 mL of TCA solution was added, the solution was left at 20 °C to facilitate precipitation for 30 min. The precipitated protein was removed by centrifugation at 4,000 g for 10 min. The remaining 2 mL of the supernatant was mixed with 0.5 mL of 2 M NaOH and the absorbance was measured directly with a UV-visible spectrophotometer (Varian Inc., USA) at a wavelength of 440 nm. Each reaction was measured in triplicate for each assay under the same conditions, and each was reported as the average activity. One unit of enzyme activity (U) was defined as the difference in the unit optical absorbance (Abs) per minute.

RESULTS AND DISCUSSION

1. CH_4 Production

The cumulative CH_4 production curves depending on the amount of FW added are shown in Fig. 1, all of which are well fitted by the modified Gompertz equation (R^2>0.99). The H_2 content in the produced biogas was negligible (<0.1%), and the fermentation process ended within 25 days. The effect of the FW addition to WAS on the total amount of CH_4 produced was crucial, and this effect gradually increased as the amount of FW added was increased. When adding 12 mL of FW, the cumulative CH_4 production was increased by 1.7 times compared to the results of a control experiment (w/o adding FW). It was suspected that the increased amount mainly originated from the FW but also somehow from WAS through a synergistic effect. The CH_4 yield in terms of the COD (the actual amount of CH_4 produced/the theoretical CH_4 production potential), indicating the energy content in the substrate that was converted to CH_4, also increased with an increase in the amount of FW added (Table 2). In addition, the FW addition resulted in an increased CH_4 production rate. This rate nearly doubled in case of adding 12 mL of FW, compared to the control.

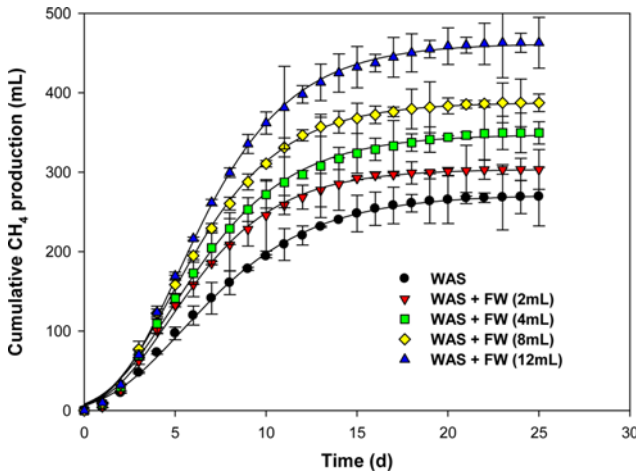

Fig. 1. Cumulative CH_4 production from waste activated sludge (WAS) and food waste (FW).

2. Synergistic Increase

In a strict definition of a synergistic effect, a certain amount of increased CH_4 production should be derived from WAS instead of being entirely derived from the FW. To reveal this clearly, a synthetic feedstock made of isotope C_{13} should be used [22]. However, we can approximate whether or not a synergistic effect existed through the theoretical approach shown in Table 3. The amount of CH_4 produced (=A) with different amounts of FW added was higher than the case of WAS alone (=B). In total, 31, 75, 116, and 190 mL more CH_4 (A-B) was produced by a FW addition. If we assume that all of the organic material contained in the added FW was converted to CH_4, the value "C" can be obtained using the following theoretical conversion: 1 g COD=350 mL CH_4. Finally, the synergistic increase in the value of "D" can be obtained by subtracting C from A-B. In all cases, the D value was positive, indicating that there was clear synergy. For example, when adding 4 mL of FW, an additional 75 mL of CH_4 was produced, of which at least 36 mL originated from WAS. It was found that at least 30% of the increased amount of CH_4 produced was from WAS in this experimental range. This represents the first report that clearly reveals this type of synergism during the anaerobic co-digestion of WAS and FW.

In some studies of co-digestion, the increased CH_4 production was attributed to a high C/N ratio with the addition of an external carbon source [5,23,24]. However, under the present experimental conditions, compared to the WAS input, a slight amount of FW was added; this only increased the total COD concentration from 20.0 to 22.2 g COD/L and the C/N ratio from 14.6 to 15.5. Therefore, it was considered that a certain important mechanism acted, playing a crucial role in the enhanced digestion efficiency of WAS. This is discussed further in the next chapter.

3. Reason of Synergism

To reveal the synergism, another experimental set comparing the control to a case in which 12 mL FW was added was also run, where not only the AD performance but also the activity levels of HEP and the ammonia concentration were measured.

As in the previous result, a 53% higher CH_4 yield and a 96% higher CH_4 production rate were achieved by the FW addition in this case

Table 3. Theoretical approach revealing the synergistic effect by food waste addition

	1	2	3	4	5
Actual CH_4 production (mL)=A	272	303	347	388	462
CH_4 production from WAS (mL)=B	-	272	272	272	272
COD added from FW (g)	-	0.06	0.11	0.22	0.33
Theoretically maximum CH_4 production from FW (mL)=C	-	20	39	78	117
Synergistic increase (mL)=A-B-C	-	11	36	38	73

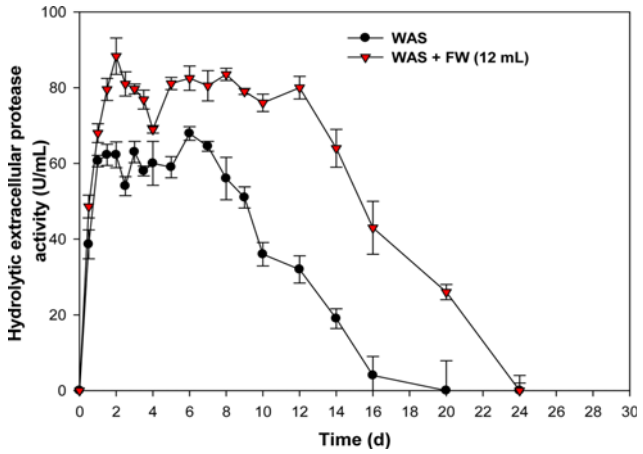

Fig. 2. Hydrolytic extracellular protease activity during anaerobic digestion of waste activated sludge and a mixture of waste activated sludge and food waste.

(data not shown). It was found that a slight FW addition significantly increased the HEP activity (Fig. 2). It jumped to 88 U/mL within two days, which was 42% higher than the level noted with the control. HEP activity of around 80 U/mL was maintained until the 12th day in the FW-added case, whereas it was only 60 U/mL and showed a noticeably decreasing trend from the 8th day in the control.

Concomitant with the HEP activity, the ammonia concentration also showed a distinctly different level between two cases (Fig. 3). In the control, the ammonia concentration gradually increased with time, reaching approximately 600 mg NH_4-N/L on the 10th day. Meanwhile, it increased to 1,000 mg NH_4-N/L within 10th days in the FW-added case. This is somewhat in contrast to previous work, which attributed the enhanced AD efficiency of WAS by co-digestion with FW to an alleviated ammonia level [25]. When the ammonia concentration change profiles were fitted by the first-order kinetics (R^2>0.98), a much higher first-rate coefficient (k) of 0.2246 (0.1485 in the control) was obtained from the FW-added case. First-order kinetics has often been used to elucidate the effect of the substrate type, temperature, and pH on AD [14,26].

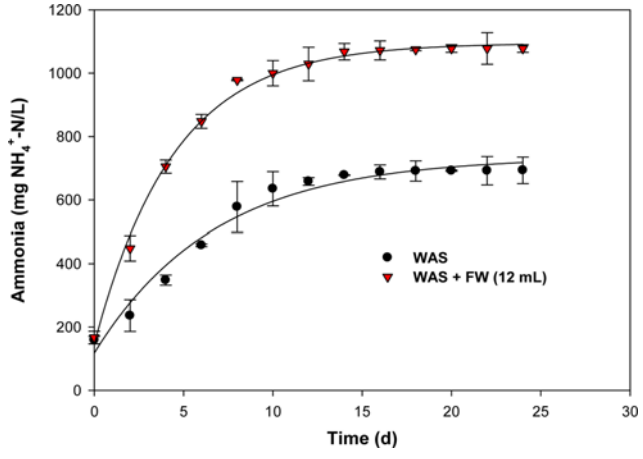

Fig. 3. Ammonia concentration profile during anaerobic digestion of waste activated sludge and a mixture of waste activated sludge and food waste.

The results above indicate that the enhanced AD efficiency of WAS caused by the addition FW is strongly related to an increased protein hydrolysis. It is a general characteristic that WAS consists of mostly protein, which has a low level of biodegradability. However, biodegradability can be enhanced when readily biodegradable organics such as FW are added, whose phenomenon is similar to co-metabolism. It is defined that a certain compound is degraded only in the presence of easily degradable organics by supplying enough energy to synthesize the new enzymes. This study clearly revealed that FW addition synergistically enhanced the AD efficiency of WAS by increasing the activity of HEP.

CONCLUSIONS

To clearly reveal the synergistic enhancement in co-digestion, a slight amount of FW was added to WAS, and BMP tests were performed. With an increase in the amount of FW added, not only the CH_4 yield but also CH_4 production rate increased. A simple calculation clearly showed synergism in that at least 30% of the increased amount of CH_4 produced was from WAS. A greater degree of HEP activity and a higher ammonia concentration were also observed in the FW-added case, supporting the contention of synergism. Consequently, the anaerobic co-digestion of WAS and FW could be more flexibly applied to the treatment of organic solid wastes to overcome the major rate-limiting step and to promote bioenergy recovery.

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NOMENCLATURE

M(t) : cumulative CH_4 production (mL) at cultivation time t (d)
P : ultimate CH_4 production [mL]
R_m : gas production rate [mL/d]
λ : lag period [d]
e : exp(1)=2.71828.
P'_0 : initial ammonia concentration [mg/L]
P′ : ammonia concentration [mg/L]
k : first-order rate coefficient
α : conversion coefficient, respectively

Abbreviation

WAS : waste activated sludge
FW : food waste
AD : anaerobic digestion
HEE : hydrolytic extracellular enzymes
HEP : hydrolytic extracellular protease
BMP : biological methane potential

COD : chemical oxygen demand

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