Relationship between the synergistic/antagonistic effect of anaerobic co-digestion and organic loading

Revised manuscript submitted to

International Biodeterioration & Biodegradation

March 2017

Tian Xie, Sihuang Xie, Muttucumaru Sivakumar, and Long D. Nghiem*

Strategic Water Infrastructure Laboratory, School of Civil Mining and Environmental Engineering, University of Wollongong, Wollongong, NSW 2522, Australia

* Corresponding author: Long Duc Nghiem, Email: longn@uow.edu.au; Ph +61 2 4221 4590

Abstract: Results from this study reveal a notable relationship between the synergistic/antagonistic performance of sewage sludge – food waste anaerobic co-digestion (AcoD) and organic loading. At the same sewage sludge content, biomethane potential (BMP) assays show an increasing specific methane yield as the content of food waste increased to the optimum organic loading of 15 kg VS/m^3. Under these conditions, the specific methane yields experimentally measured in this study were considerably higher than those calculated by adding the specific methane individual co-substrates during mono-digestion. On the other hand, at above the optimum organic loading value, the antagonistic effect (i.e. lower specific methane yield compared to mono-digestion) was observed. The relationship between synergistic performance of AcoD and organic loading was also evidenced in the removal of volatile solids as well as chemical oxygen demand. Further analysis of the intermediate products show that methanogenesis was the rate limiting step during AcoD at a high organic loading value. As the organic loading increased, the digestion lag phase increased and the hydrolysis rate decreased.
Keywords: Anaerobic co-digestion; synergistic effects; organic loading; sewage sludge; food waste; energy recovery.

1. Introduction

Sewage sludge is a solid by-product from municipal wastewater treatment. Because sewage sludge is rich in biodegradable organics and pathogenic agents, adequate treatment is necessary prior to disposal or any form of land applications (Semblante et al., 2014). Given the large amount of sewage sludge generated each day, sewage sludge management has become a major issue for the wastewater industry. Indeed, the treatment and disposal cost of sewage sludge accounts for up to 50% of the total operational budget of a typical wastewater treatment plant (WWTP) (Appels et al., 2008; Li et al., 2014).

Anaerobic digestion (AD) is the most widely used technology for sewage sludge treatment. AD is a multi-stage biological process to convert organic materials to biogas and stabilised biosolids in the absence of oxygen (Mata-Alvarez et al., 2014). Biogas contains 40-60% CH_4, 30-40% CO_2, and a trace amount of other gases such as H_2S and water vapour (Chynoweth et al., 2001; Wickham et al., 2016). Given its methane content, biogas is a valuable renewable fuel, which can be used by a combined heat and power engine to generate electricity to offset part of the energy demand at the WWTP and heat which can be used by the AD process itself

(Shen et al., 2015). Stabilised biosolids is also a valuable resource and can be used for agriculture production and soil reclamation (Armstrong et al., In Press).

The role of AD has become even more significant given the recent paradigm shift toward a circular economy in which sludge and organic wastes can be utilised as a renewable resource of energy and nutrients through anaerobic co-digestion (AcoD) (Mata-Alvarez et al., 2014; Nghiem et al., 2017). AcoD can utilise the infrastructure at existing WWTPs without a major capital investment (Nghiem et al., 2017). A significant increase in methane production can be achieved when the mixture of substrates has a balanced composition of carbon source, nutrients, and trace elements (Panpong et al., 2014b). The economic benefits from AcoD can be realised through gate fee revenue from organic wastes and bioenergy generation (Xie et al., 2016). In terms of environmental benefits, AcoD can divert the organic waste from the landfills and eliminate the greenhouse gas emissions at the same time (Nghiem et al., 2014; Xie et al., 2016). Other benefits include the dilution of toxic compounds, improve nutrition balance, and load increase of the biodegradable organic matter (Sosnowski et al., 2003).

A range of organic wastes is available for AcoD operation. Among them, food waste is arguably the most abundant substrate that is also rich in energy (i.e. carbon) and nutrient content (Thi et al., 2016). In general, food waste consists of 10-30% readily biodegradable organic materials (Ratanatamskul & Manpetch, 2016; Zhang et al., 2016; Zhang et al., 2007). Given the high organic content of food waste, AD has been identified as an ideal solution for energy recovery from food waste. In addition to the many benefits of AcoD discussed above, there have been several reports of the synergistic effect when sewage sludge is co-digested with organic-rich substrates, particularly food waste (Fernández et al., 2005; Khairuddin et al., 2015; Panpong et al., 2014a; Xie et al., 2017). This synergistic effect is defined as an increase methane yield compared to mono-digestion by per unit VS or COD input. However, data currently available in the literature are rather inconsistent. Antagonistic and neutral effects have also been observed during AcoD of sewage sludge and organic wastes. Silvestre et al. (2014) reported a decrease in methane production by more than 40% during thermophilic AcoD of sewage sludge and grease waste when the content of grease waste increased from 27 to 37% at the same organic loading. Their results demonstrate an antagonistic effect possibly due to fatty acid inhibition (Silvestre et al., 2014). In another study, Silvestre et al. (2015) did not observe any changes in the specific methane yield during mesophilic AcoD of sewage sludge and crude glycerol at more than 1% (v/v) co-substrate addition. Given the inconsistency in the literature regarding synergistic effect during AcoD, it is hypothesised here that organic loading can play a major role in governing the specific methane yield.

In practice, organic loading is a key parameter in the continuous operation of AcoD (Mata-Alvarez et al., 2014). In a batch process, organic loading can be defined as the ratio of either VS or COD content over volume. In a continuous process, the retention time is taken into account and the organic loading rate (OLR) can be used instead. Mono-digestion of sewage sludge at WWTPs is usually operated at an OLR of less than 1 kg VS/(m^3.d) (Nghiem et al., 2017). On the other hand, given the high organic content of the co-substrate (particularly food waste), AcoD is operated at a much higher OLR value of up to 4.6 kg VS/(m^3.d) (Nghiem et al., 2017; Zhang & Jahng, 2012), which may result in operational stability issues. Therefore, in terms of treatment efficiency and process stability, many dedicated efforts have been devoted to exploring the optimum organic loading for AcoD operation (Agyeman & Tao, 2014; Aramrueang et al., 2016; Li et al., 2015; Paudel et al., In Press).

The aim of this study is to explore the relationship between organic loading and the synergistic effects during AcoD of sewage sludge and food waste through BMP evaluation. The specific objectives include (i) evaluating the process performance and stability from total solids (TS), VS, and soluble COD removal, (ii) determining the hydrolysis rate constant (K_h) based on the reaction kinetics, (iii) appraising the biomethane yield and the synergistic effect at various organic loadings.

2. Materials and methods

2.1 Substrate characterization

Digestate and primary sludge samples were obtained from a full-scale WWTP in Wollongong and used as the inoculum and substrate respectively. Adult dog food from Optimum was used to simulate food waste. The Optimum dog food (beef & rice) contains mainly protein, carbohydrate, and fat. All substrates and inoculum were stored at 4 °C for less than 3 days prior to the BMP evaluation.

2.2 BMP assays

Food waste and sewage sludge were co-digested using a custom-built BMP system. The BMP system consisted of an array of 1000 mL volume fermentation glass bottles (Wiltronics

Research Pty Ltd) and gas collection galleries as shown in Figure 1 (Nghiem et al., 2014). Each bottle was submerged in a water bath (Model SWB20D, Ratek Instrument Pty Ltd) which constantly maintained the temperature at 35.0 ± 0.1 °C. Each setup of fermentation bottle consisted of a rubber stopper, S-shaped airlock, and soft tubes, which connect to a gas valve to the gas collection gallery and sampling valve for taking samples. The S-shaped airlock can maintain the substrates under an anaerobic condition by allowing the releasement of biogas produced in the fermentation bottle while preventing any intrusion of air into the system. The gas collector consists of a 1000 mL volume plastic cylinder and a plastic container, which both filled up with 1 M sodium hydroxide solution to ensure the gathered biomethane free from the disturbance of carbon dioxide and hydrogen sulphide.

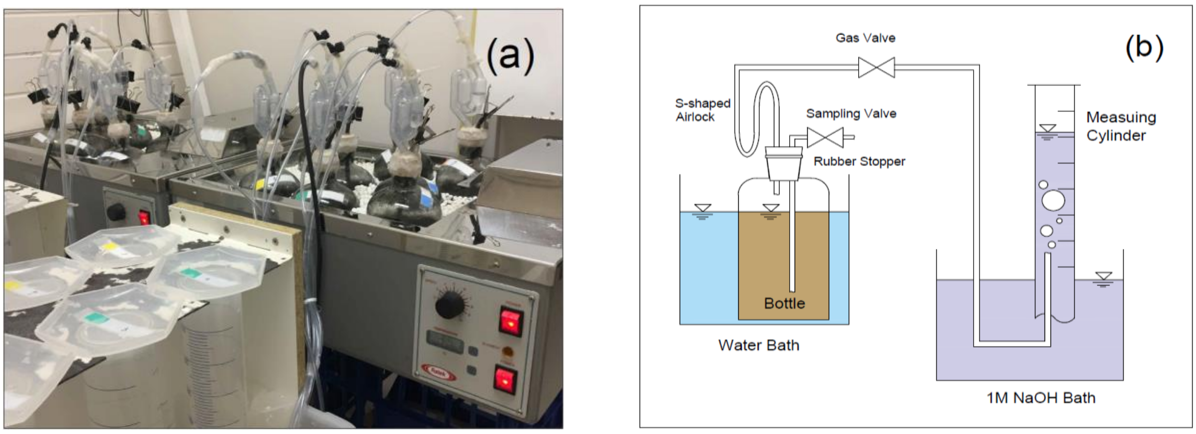

Figure 1. (a) Photograph and (b) Schematic diagram of the BMP test equipment including water bath, BMP bottle, and gas collection gallery

Prior to the BMP evaluation, all the fermentation bottles were flushed with N_2 for 5 min before the immediate filling of co-substrates and inoculum as introduced in section 2.1. Organic loading was calculated based on the initial VS content in each BMP bottle (Table 1). All BMP experiments were conducted in duplicate.

Two BMP bottles were filled with only inoculum and used as the reference. Mono-digestion was simulated by filling the BMP bottles with inoculum and either sewage sludge or food waste. Co-digestion was simulated by filling the BMP bottles with inoculum, sewage sludge, and food waste. The active volume of all BMP bottles was 750 mL, which consisted of 450 mL of inoculum and a specified amount of substrate as noted in Table 1. When the substrate volume was less than 300 mL, Milli-Q water was added to obtain the total volume of 750 mL.

After filling with inoculum and substrates, the BMP bottles were flushed with N_2 again, sealed with rubber stopper instantly, and placed in the water bath, which was maintained at 35 °C. The gas valves were then opened to allow biogas from entering to the gas collection gallery. The BMP experiments were terminated when the daily methane production during three consecutive days was less than 10 mL. All BMP bottles were mixed manually twice a day.

The BMP protocol used in this study is broadly consistent with the standard procedure recommended by Holliger et al., (Holliger et al., 2016). However, it is noted that in this study, the inoculum to substrate (I/S) ratio was not constant to simulate varying organic loading at a constant reactor volume.

Table 1. Operating conditions of batch experiments with 450 mL inoculum and the total volume of 750 mL.

	Mono-digestion		Co-digestion			
	SS	FW_{20}	FW_{30} + SS	FW_{70} + SS	FW_{110} + SS	FW_{150} + SS
Organic loading (kg VS/m^3)	5.67	3.56	8.17	15.29	22.4	29.52
I/S ratio	1.53:1	2.44:1	1.06:1	0.57:1	0.39:1	0.29:1

SS: sewage sludge (300g); FW_{20}: 20 g food waste; FW_{30} + SS: 30 g food waste and 150 g sewage sludge; FW_{70} + SS: 70 g food waste and 150 g sewage sludge; FW_{110} + SS: 110 g food waste and 150 g sewage sludge; FW_{150} + SS: 150 g food waste and 150 g sewage sludge.

2.3 First order kinetics

2.3.1 Biomethane production

Methane productivity was calculated and the cumulative methane yield was simulated with modified Gompertz model in Eq. (1):

$$M = Pexp\{-exp\left[\frac{eR_{max}(\lambda - t)}{P} + 1\right]\} \quad (1)$$

Where P is the maximum methane potential (mL); M is the cumulative methane production (mL); R_{max} is the maximum methane production rate (mL/d); λ is the lag phase (d); e is Euler's number (≈2.71828); and t is the time (d).

2.3.2 Hydrolysis process

K_h reflects the rate of the hydrolysis stage and depends highly on the addition of co-substrate, and operating conditions (Xie et al., 2017). It can be directly calculated using the net cumulative biogas yield by applying the equation as follows:

$$Ln\left(\frac{P-M}{P}\right) = -K_h t \quad (2)$$

Non-linear fitting of the biomethane production based on Eq. (1) and linear regression of Ln[(P-M)/P] against time (t) based on Eq. (2) were conducted using the IBM SPSS software package (version 23.0) to determine λ and K_h, respectively. Eq. (2) is based on the assumption that hydrolysis is the limiting step and all COD was converted to methane. Thus, in this study, K_h was obtained from the initial period when the accumulation of COD has not occurred. The p-value less than 0.05 is considered statistically significant.

2.4 Analytical methods

Liquid sample of 1 mL was taken from each BMP bottle periodically using a 5-mL syringe. All the samples were stored at 4 °C to avoid further digestion in the samples. The total volume of these taken samples occupied less than 1.5% of the initial total volume to minimise the impact of further digestion performance in the BMP bottle. Samples were diluted to 5 mL and 10 mL respectively for the pH and total COD measurements. The dilution factor was taken into account to back calculate the actual pH value of the initial sample. After pH and total COD measurements, samples were further diluted to a total volume of 30 mL followed by centrifuging at 3750 rpm for 20 min. Then, the supernatant of 15 mL from each sample was taken and stored at 4 °C for soluble COD and total organic acid (TOA) analysis. Total and soluble COD were measured by a Hach DBR200 COD Reactor and a Hach DR/2000 spectrophotometer (program number 435 COD HR) according to US-EPA Standard Method 5220. Biomethane production was recorded at 10 am and 5 pm each day by reading the displacement volume in the gas collection cylinder. The detailed method for measuring methane yield was explained in Wickham et al. (2016). TS and VS were measured by following the standard method 2540G (Eaton et al., 2005) within 3 days of sample collections. TOA was conducted according to the standard distillation method 5560C (Eaton et al., 2005).

2.5 Specific methane yield and removal rate

Thus, the specific methane yield was calculated as:

$$Y_{sp} = \frac{(Y_{sub} - Y_{in})}{VS_{added}} \quad (3)$$

Where Y_{sp} is the specific methane yield (mL); Y_{sub} is the total methane production from the substrate (mL); Y_{in} is the total methane yield from the inoculum, which was 1145 mL, and VS_{added} is the mass VS added from the substrate in the BMP bottle (g).

The calculated methane yield from a mixture of sewage sludge and food waste could also be obtained from the specific methane yield of each individual substrate without taking into account any synergistic effect:

$$Y_{cp} = \frac{VS_{FW} \times Y_{FW} + VS_{SS} \times Y_{SS}}{VS_{FW} + VS_{SS}} \quad (4)$$

Where Y_{cp} is the calculated methane yield (mL methane/g VS_{added}); VS_{FW} is the VS added from the food waste in the co-digestion BMP bottles (g); Y_{FW} is the specific methane yield of mono-digestion of 20 g food waste (mL methane/g VS_{added}); VS_{SS} is the VS added from the sewage sludge in the co-digestion bottles; and Y_{SS} is the specific methane yield of mono-digestion of sewage sludge (mL methane/g VS_{added}).

The removal rate can be calculated using the following equation (Xie et al., 2017):

$$Removal = 100\% \times \left(1 - \frac{C_{Co,End} - C_{In,End}}{C_{Co,Ini} - C_{In,End}}\right) \quad (5)$$

Where $C_{Co,End}$ is the concentration of the substrates in the BMP bottles at the end of the experiment; $C_{In,End}$ is the concentration of inoculum in controls at the ending point; $C_{Co,Ini}$ refers to the initial concentration in the co-digestion bottles.

3. Results and discussion

3.1 Substrate characterization

Table 2. Key properties of inoculum, primary sludge, and food waste.

	Inoculum	Primary Sludge	Food Waste
TS (%)	2.18 ± 0.01	1.91 ± 0.26	19.69 ± 1.05
VS (%)	1.45 ± 0.03	1.42 ± 0.19	13.34 ± 2.90
VS/TS (%)	66.52	74.14	67.73
pH	7.28 ± 0.01	5.80 ± 0.07	6.44 ± 0.01
Total COD (mg/kg)	16,100 ± 950	21,300 ± 1350	798,000 ± 38,184
Soluble COD (mg/kg)	1,120 ± 66	1,800 ± 114	93,000 ± 2,828

Food waste exhibited distinctive properties compared to sewage sludge in terms of pH, COD and TS/VS (Table 2). Although the VS/TS ratio of food waste was comparable to that of sewage sludge, the VS content of food waste was approximately 10 times higher than that of primary sludge. Most notably, the soluble COD of food waste was almost 40 times higher than that of primary sludge. The results suggest that much of the organic content of food waste is readily biodegradable. The inoculum showed a neutral pH. On the other hand, sewage sludge was slightly acidic, indicating some initial hydrolysis of sewage sludge (Wickham et al., 2016; Xie et al., 2017). Food waste was also slightly acidic because of the presence of mainly short-chain acids (Beck-Friis et al., 2001; Sundberg et al., 2004).

3.2 Effects of organic loading on specific methane yields

Figure 2 shows cumulative methane yield from each BMP test as a function of time and the influence of organic loading on specific methane yields. Lag phase can be observed at high organic loading. It is also apparent that duration of the observed lag phase increased as the organic loading increased. In addition, there appears to be an optimum organic loading at approximately 15 kg VS/m^3, corresponding to the co-digestion of 70 g of food waste and 150 g of sewage sludge. At above this value, organic overloading occurred, evidenced by excessive lag time and insignificant specific methane yields (Figure 2).

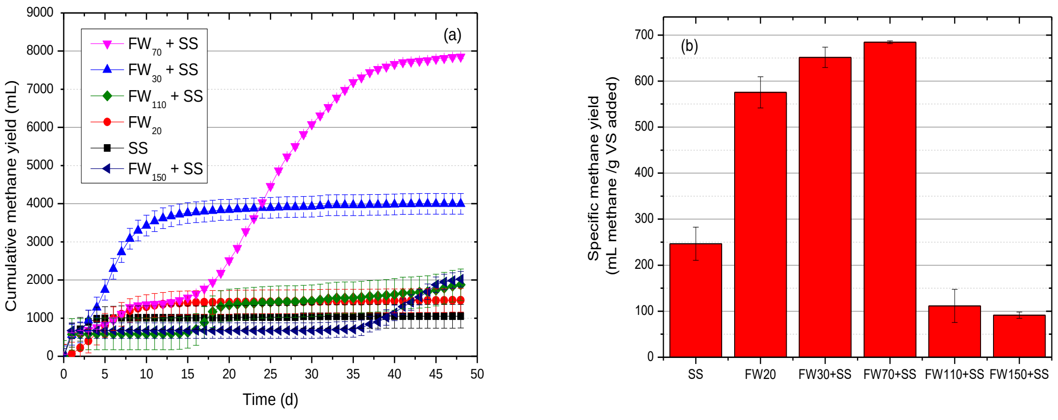

Figure 2. (a) Cumulative methane yield as a function of time and (b) Specific methane yield at day 48 over various organic loadings. SS: sewage sludge (300g); FW$_{20}$: 20 g food waste; FW$_{30}$ + SS: 30 g food waste and 150 g sewage sludge; FW$_{70}$ + SS: 70 g food waste and 150 g sewage sludge; FW$_{110}$ + SS: 110 g food waste and 150 g sewage sludge; FW$_{150}$ + SS: 150 g food waste and 150 g sewage sludge.

Results presented in Figure 2 also show clear evidence of the synergistic effect of co-digestion. Notably higher specific methane yield from the co-digestion between food waste and sewage sludge at organic loadings of 8 and 15 kg VS/m^3, corresponding to FW_{30} + SS and FW_{70} + SS, can be seen in Figure 2 compared to mono-digestion of only food waste. The total methane production for 30 g and 70 g food waste co-digestion bottles were 3990 mL and 7850 mL, respectively. By comparison, the total methane production from mono-digestion of sewage sludge and food waste were 1050 mL and 1470 mL. After normalising by the amount of VS in each BMP test, the specific methane yield increased as the organic loading increased up to the optimum value of 15 kg VS/m^3. These results demonstrate the dependence of the synergistic effect of co-digestion on organic loading.

Table 3. Measured and calculated specific methane yield (mL methane/g VS_{added}) at various organic loadings.

	Mono-digestion		Co-digestion			
	SS	FW_{20}	FW_{30} + SS	FW_{70} + SS	FW_{110} + SS	FW_{150} + SS
Organic loading (kg VS/m^3)	5.67	3.56	8.17	15.29	22.4	29.52
Measured specific methane yield	246.5	575.4	651.5	684.5	111.5	91.4
Calculated specific methane yield	246.5	575.4	461.3	514.4	533.8	543.8

SS: sewage sludge (300g); FW_{20}: 20 g food waste; FW_{30} + SS: 30 g food waste and 150 g sewage sludge; FW_{70} + SS: 70 g food waste and 150 g sewage sludge; FW_{110} + SS: 110 g food waste and 150 g sewage sludge; FW_{150} + SS: 150 g food waste and 150 g sewage sludge.

Further evidence of the synergistic effect of food waste and sewage sludge co-digestion as well as the dependence of the synergistic effect of co-digestion on organic loading can also be seen in Table 3. The specific methane yield of co-digestion between sewage sludge with either 30 or 70 g experimentally obtained in this study was 30-40% higher than the calculated value from mono-digestion of each individual substrate by ignoring the synergistic effect (Eq. 4). Organic loading is a major factor under these experimental circumstances. It is noted that I/S ratio and pH may also impact the specific methane yields (Hashimoto, 1989; Jayaraj et al., 2014). By contrast, antagonistic effect was observed for 110 g and 150 g food waste co-digestion with sewage sludge due to organic overloading. In these two BMP tests, due to organic overloading, the specific methane yield was even lower than that from mono-digestion (section 3.3.3). A similar phenomenon was reported in a continuous system and the specific methane yield decreased by 25% when increased the OLR from 2 to 3 kg VS/(m^3.d) (Xie et al., 2012). In terms of microorganism communities, organic overloading has been a major inhibitory impact on the methanogenic communities (Regueiro et al., 2015). Under an overloading condition, excessive organic acids can accumulate in the system. Both methanogenic population and the *Syntrophomonadaceae* family, which has been identified with the syntrophic relationship to methanogenic Archaea, decreased significantly due to the accumulation of volatile fatty acids (Kleyböcker et al., 2014; Regueiro et al., 2015). Hence, a retention time much longer than the period of 48 days in this study would be required to evaluate the specific methane yield (Holliger et al., 2016).

3.3 System performance and stability

3.3.1 Intermediate product parameters

System performance and stability can be evaluated by examining intermediate product parameters including soluble COD and TOA as well as pH value of the digestate. The pH profile during the entire digestion process is presented in Figure 3a. Subjected to the limited buffering capacity, pH decreased significantly due to the fast accumulation of the intermediate acids in hydrolysis and acidogenesis phases. Once the acid production has been exhausted and the methanogenic process was able to convert organic acid to methane gas, the pH was recovered to a neutral value. It is noteworthy that pH dropped more rapidly and significantly for BMP bottles with high organic loading. This observation can be attributed to the accumulation of intermediate acids due to the slow reaction rate in methanogenesis phase. Under this circumstance, the methanogenesis process is considered to be the rate-limiting step (Ma et al., 2013). The exceedingly accumulated intermediate acids, on the other hand, led to a longer microbe adaptation time, which is a longer lag phase. For BMP bottles with low (8 kg VS/m^3) or optimal (15 kg VS/m^3) organic loading, no observable inhibition was observed. The pH value decreased but rapidly recovered to neutral (Figure 3).

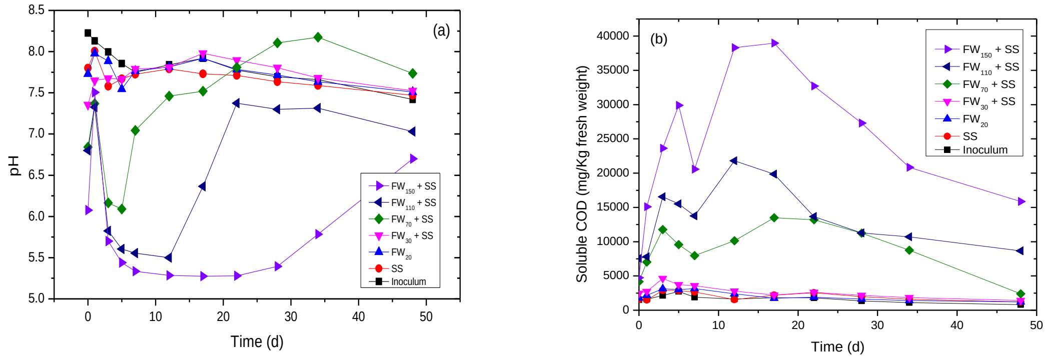

Figure 3. (a) pH and (b) soluble COD concentrations as a function of time in the BMP tests. SS: sewage sludge (300g); FW_{20}: 20 g food waste; FW_{30} + SS: 30 g food waste and 150 g sewage sludge; FW_{70} + SS: 70 g food waste and 150 g sewage sludge; FW_{110} + SS: 110 g food waste and 150 g sewage sludge; FW_{150} + SS: 150 g food waste and 150 g sewage sludge.

Soluble COD content in the BMP bottle increased due to the accumulation of organic acids. A similar observation can be seen with TOA content in all BMP bottles (data not shown). As noted above, the methanogenesis phase is the rate-limiting step for bottles with high organic loadings. The highest soluble COD content (38,970 mg/L) was observed in the 150 g food waste and sewage sludge bottles (Figure 3b). On the other hand, soluble COD and TOA contents were low and stable at low organic loading. In this case, hydrolysis could be considered as the rate-limiting step. It is noteworthy that soluble COD fluctuated in the first 7 days of the reaction for BMP bottles with organic loading higher than 15 kg VS / m^3. It may be the result of a different hydrolysis rate between readily and slowly biodegradable organics.

3.3.2 Gompertz modelling

Table 4. Performance of mono- and co-digestion with sewage sludge and food waste.

	Mono-digestion		Co-digestion	
	SS	FW_{20}	FW_{30} + SS	FW_{70} + SS
P (mL)	1536.0 ± 4.0	1555.7 ± 2.0	3960.5 ± 13.0	8956.4 ± 174.0
R_{max} (mL methane/d)	372.9 ± 11.6	227.9 ± 2.5	500.3 ± 12.2	338.0 ± 18.9
Lag phase, λ (day)	0.4 ± 0.1	1.1 ± 0.041	1.6 ± 0.1	9.8 ± 0.7
Ultimate specific methane yield (mL CH_4/g VS_{added})	330.7	557.7	591.8	715.6
R^2	0.99	0.99	0.99	0.98

SS: sewage sludge (300g); FW_{20}: 20 g food waste; FW_{30} + SS: 30 g food waste and 150 g sewage sludge; FW_{70} + SS: 70 g food waste and 150 g sewage sludge; FW_{110} + SS: 110 g food waste and 150 g sewage sludge; FW_{150} + SS: 150 g food waste and 150 g sewage sludge.

The modified Gompertz model was used to simulate the digestion process. As noted in section 2.3.1, the lag phase (λ) and the ultimate specific methane yield could be obtained by fitting data presented in Figure 2 to the Gompertz model. The ultimate specific methane yields obtained from the Gompertz model (Table 4) were consistent with experimentally obtained values previously presented in Table 3. Similar results at a comparable organic loading level have also been reported by Xie et al. (2017).

Table 4 also shows an increasing lag phase as the organic loading increased. The lag phase during mono-digestion of sewage sludge and food waste was insignificant. For comparison, a lag phase of 9.8 days was observed at the optimum organic loading of 15 kg VS / m^3 (70 g of food waste and 150 g of sewage sludge). In the lab scale, a similar lag phase expansion was observed by Kougias et al. (2014) when increased the organic proportion in the feeding substrate.

3.3.3 TS, VS, and soluble COD removals

The removals for TS, VS and soluble COD are important properties in the batch system experiment, which can be used to evaluate the performance of the digestion process. The soluble COD removal represents the reduction of soluble organic content.

Table 5. TS, VS, and Soluble COD removals at various organic loadings.

Removal (%)	Mono-digestion		Co-digestion			
	SS	FW_{20}	FW_{30} + SS	FW_{70} + SS	FW_{110} + SS	FW_{150} + SS
TS	76.2	98.4	82.3	82.9	64.3	55.2
VS	67.8	94.3	72.4	75.6	56.0	40.9
Soluble COD	48.0	59.7	62.4	53.4	-16.5%	-283.9%

SS: sewage sludge (300 g); FW_{20}: 20 g food waste; FW_{30} + SS: 30 g food waste and 150 g sewage sludge; FW_{70} + SS: 70 g food waste and 150 g sewage sludge; FW_{110} + SS: 110 g food waste and 150 g sewage sludge; FW_{150} + SS: 150 g food waste and 150 g sewage sludge.

TS, VS and soluble COD removals in the co-digestion bottles were higher than those in the mono-digestion of sewage sludge. These results provide further evidence of the synergistic effect of co-digestion and the biodegradable nature of food waste during AD (Grimberg et al., 2015). It is noted that the production and consumption of soluble COD can occur simultaneously, thus, data in Table 5 represent the overall balance of soluble COD in the system. The low removal of soluble COD during co-digestion of food waste and sewage sludge can be attributed to the very high soluble COD content in food waste as previously discussed in section 3.1.

3.4 Kinetics of the hydrolysis process

Table 6. Hydrolysis rate constant for mono- and co-digestion of sewage sludge and organic waste.

	Mono-digestion		Co-digestion	
	SS	FW_{20}	FW_{30} + SS	FW_{70} + SS
K_h	0.458	0.263	0.202	0.123
R^2	0.978	0.965	0.990	0.992
p-value	0.001	0.001	0.001	0.001

SS: sewage sludge (300 g); FW_{20}: 20 g food waste; FW_{30} + SS: 30 g food waste and 150 g sewage sludge; FW_{70} + SS: 70 g food waste and 150 g sewage sludge; FW_{110} + SS: 110 g food waste and 150 g sewage sludge; FW_{150} + SS: 150 g food waste and 150 g sewage sludge.

The K_h of the hydrolysis process was determined using Eq. (2) and cumulative methane production data presented in Figure 2a. K_h decreased as the organic loading increased (Table 6). In other words, the hydrolysis rate decreased with increasing organic content. These results are consistent with data reported by Wirth et al. (2015) and Cheng et al. (2016). These results are also consistent with the increasing lag phase at increasing organic loading as discussed in section 3.3.2. The observed decrease in K_h value as the amount of food waste increased from 30 to 110 g indicates the need to enhance the hydrolysis process during co-digestion possible by an additional acid phase digester (Koch et al., 2015).

4. Conclusion

This study shows that the synergistic/antagonistic performance of AcoD between sewage sludge and food waste was dependent on organic loading. At the same sewage sludge content, the specific methane yield increased as the content of food waste increased to the optimum organic loading of 15 kg VS/m^3. At or below this optimum organic loading, the experimentally obtained specific methane yields were notably higher than those values calculated by adding the specific methane yields of individual co-substrates during mono-digestion. On the other hand, at an excessive organic loading value, the antagonistic effect (i.e. lower specific methane yield compared to mono-digestion) was observed. The interplay between synergistic performance of AcoD and organic loading could also be seen in the removal rates of VS as well as COD. Results from intermediate product analysis also suggests that methanogenesis was the rate limiting step during AcoD.

5. Acknowledgement

This research was supported under Australian Research Council's Linkage Project funding scheme (project LP150100304).

6. References

Agyeman, F.O., Tao, W. 2014. Anaerobic co-digestion of food waste and dairy manure: Effects of food waste particle size and organic loading rate. *Journal of Environmental Management*, **133**, 268-274.

Appels, L., Baeyens, J., Degrève, J., Dewil, R. 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science*, **34**(6), 755-781.

Aramrueang, N., Rapport, J., Zhang, R. 2016. Effects of hydraulic retention time and organic loading rate on performance and stability of anaerobic digestion of Spirulina platensis. *Biosystems Engineering*, **147**, 174-182.

Armstrong, D.L., Rice, C.P., Ramirez, M., Torrents, A. In Press. Influence of thermal hydrolysis-anaerobic digestion treatment of wastewater solids on concentrations of triclosan, triclocarban, and their transformation products in biosolids. *Chemosphere*.

Beck-Friis, B., Smårs, S., Jönsson, H., Kirchmann, H. 2001. SE—Structures and Environment. *Journal of Agricultural Engineering Research*, **78**(4), 423-430.

Cheng, Q., Chen, Z., Deng, F., Liao, Y., Xiao, B., Li, J. 2016. Kinetic evaluation on the degradation process of anaerobic digestion fed with piggery wastewater at different OLRs. *Biochemical Engineering Journal*, **113**, 123-132.

Chynoweth, D.P., Owens, J.M., Legrand, R. 2001. Renewable methane from anaerobic digestion of biomass. *Renewable Energy*, **22**(1–3), 1-8.

Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E. 2005. *Standard methods for the examination of water and wastewater*. APHA-AWWA-WEF, Washington, D.C.

Fernández, A., Sánchez, A., Font, X. 2005. Anaerobic co-digestion of a simulated organic fraction of municipal solid wastes and fats of animal and vegetable origin. *BEJ Biochemical Engineering Journal*, **26**(1), 22-28.

Grimberg, S.J., Hilderbrandt, D., Kinnunen, M., Rogers, S. 2015. Anaerobic digestion of food waste through the operation of a mesophilic two-phase pilot scale digester – Assessment of variable loadings on system performance. *Bioresource Technology*, **178**, 226-229.

Hashimoto, A.G. 1989. Effect of inoculum/substrate ratio on methane yield and production rate from straw. *Biological Wastes*, **28**(4), 247-255.

Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S., Baier, U., Bougrier, C., Buffière, P., Carballa, M., de Wilde, V., Ebertseder, F., Fernández, B., Ficara, E., Fotidis, I., Frigon, J.C., de Laclos, H.F., Ghasimi, D.S.M., Hack, G., Hartel, M., Heerenklage, J., Horvath, I.S., Jenicek, P., Koch, K., Krautwald, J., Lizasoain, J., Liu, J., Mosberger, L., Nistor, M., Oechsner, H., Oliveira, J.V., Paterson, M., Pauss, A., Pommier, S., Porqueddu, I., Raposo, F., Ribeiro, T., Rüsch Pfund, F., Strömberg, S., Torrijos, M., van Eekert, M., van Lier, J., Wedwitschka, H., Wierinck, I. 2016. Towards a standardization of biomethane potential tests. *Water Science and Technology*, **74**(11), 2515-2522.

Jayaraj, S., Deepanraj, B., Sivasubramanian, V. 2014. Study on the effect of pH on biogas production from food waste by anaerobic digestion. *Proceedings of the 9th International Green Energy Conference, Tianjin, China, May*. pp. 25-26.

Khairuddin, N., Manaf, L.A., Halimoon, N., Ghani, W.A.W.A.K., Hassan, M.A. 2015. High Solid Anaerobic Co-digestion of Household Organic Waste with Cow Manure. *Procedia Environmental Sciences*, **30**, 174-179.

Kleyböcker, A., Lienen, T., Liebrich, M., Kasina, M., Kraume, M., Würdemann, H. 2014. Application of an early warning indicator and CaO to maximize the time–space-yield of an completely mixed waste digester using rape seed oil as co-substrate. *Waste Management*, **34**(3), 661-668.

Koch, K., Helmreich, B., Drewes, J.E. 2015. Co-digestion of food waste in municipal wastewater treatment plants: Effect of different mixtures on methane yield and hydrolysis rate constant. *Applied Energy*, **137**, 250-255.

Kougias, P.G., Kotsopoulos, T.A., Martzopoulos, G.G. 2014. Effect of feedstock composition and organic loading rate during the mesophilic co-digestion of olive mill wastewater and swine manure. *Renewable Energy*, **69**, 202-207.

Li, D., Liu, S., Mi, L., Li, Z., Yuan, Y., Yan, Z., Liu, X. 2015. Effects of feedstock ratio and organic loading rate on the anaerobic mesophilic co-digestion of rice straw and cow manure. *Bioresource Technology*, **189**, 319-326.

Li, X., Dai, X., Takahashi, J., Li, N., Jin, J., Dai, L., Dong, B. 2014. New insight into chemical changes of dissolved organic matter during anaerobic digestion of dewatered sewage sludge using EEM-PARAFAC and two-dimensional FTIR correlation spectroscopy. *Bioresource Technology*, **159**, 412-420.

Ma, J., Frear, C., Wang, Z.-w., Yu, L., Zhao, Q., Li, X., Chen, S. 2013. A simple methodology for rate-limiting step determination for anaerobic digestion of complex substrates and effect of microbial community ratio. *Bioresource Technology*, **134**, 391-395.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M., Fonoll, X., Peces, M., Astals, S. 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renewable and Sustainable Energy Reviews*, **36**, 412-427.

Nghiem, L.D., Koch, K., Bolzonella, D., Drewes, J.E. 2017. Full scale co-digestion of wastewater sludge and food waste: Bottlenecks and possibilities. *RSER Renewable and Sustainable Energy Reviews*, **72**, 354-362.

Nghiem, L.D., Nguyen, T.T., Manassa, P., Fitzgerald, S.K., Dawson, M., Vierboom, S. 2014. Co-digestion of sewage sludge and crude glycerol for on-demand biogas production. *International Biodeterioration & Biodegradation*, **95, Part A**, 160-166.

Panpong, K., Srisuwan, G., O-Thong, S., Kongjan, P. 2014a. Anaerobic Co-digestion of Canned Seafood Wastewater with Glycerol Waste for Enhanced Biogas Production. *Energy Procedia*, **52**, 328-336.

Panpong, K., Srisuwan, G., O-Thong, S., Kongjan, P. 2014b. Enhanced Biogas Production from Canned Seafood Wastewater by Co-digestion with Glycerol Waste and Wolffia Arrhiza. *EGYPRO Energy Procedia*, **52**, 337-351.

Paudel, S., Kang, Y., Yoo, Y.-S., Seo, G.T. In Press. Effect of volumetric organic loading rate (OLR) on H2 and CH4 production by two-stage anaerobic co-digestion of food waste and brown water. *Waste Management*.

Ratanatamskul, C., Manpetch, P. 2016. Comparative assessment of prototype digester configuration for biogas recovery from anaerobic co-digestion of food waste and rain tree leaf as feedstock. *International Biodeterioration & Biodegradation*, **113**, 367-374.

Regueiro, L., Lema, J.M., Carballa, M. 2015. Key microbial communities steering the functioning of anaerobic digesters during hydraulic and organic overloading shocks. *Bioresource Technology*, **197**, 208-216.

Semblante, G.U., Hai, F.I., Ngo, H.H., Guo, W., You, S.-J., Price, W.E., Nghiem, L.D. 2014. Sludge cycling between aerobic, anoxic and anaerobic regimes to reduce sludge production during wastewater treatment: Performance, mechanisms, and implications. *Bioresource Technology*, **155**, 395-409.

Shen, Y., Linville, J.L., Urgun-Demirtas, M., Mintz, M.M., Snyder, S.W. 2015. An overview of biogas production and utilization at full-scale wastewater treatment plants (WWTPs) in the United States: challenges and opportunities towards energy-neutral WWTPs. *Renewable and Sustainable Energy Reviews*, **50**, 346-362.

Silvestre, G., Fernández, B., Bonmatí, A. 2015. Addition of crude glycerine as strategy to balance the C/N ratio on sewage sludge thermophilic and mesophilic anaerobic co-digestion. *Bioresource Technology*, **193**, 377-385.

Silvestre, G., Illa, J., Fernández, B., Bonmatí, A. 2014. Thermophilic anaerobic co-digestion of sewage sludge with grease waste: Effect of long chain fatty acids in the methane yield and its dewatering properties. *Applied Energy*, **117**, 87-94.

Sosnowski, P., Wieczorek, A., Ledakowicz, S. 2003. Anaerobic co-digestion of sewage sludge and organic fraction of municipal solid wastes. *Advances in Environmental Research*, **7**(3), 609-616.

Sundberg, C., Smårs, S., Jönsson, H. 2004. Low pH as an inhibiting factor in the transition from mesophilic to thermophilic phase in composting. *Bioresource Technology*, **95**(2), 145-150.

Thi, N.B.D., Lin, C.-Y., Kumar, G. 2016. Waste-to-wealth for valorization of food waste to hydrogen and methane towards creating a sustainable ideal source of bioenergy. *Journal of Cleaner Production*, **122**, 29-41.

Wickham, R., Galway, B., Bustamante, H., Nghiem, L.D. 2016. Biomethane potential evaluation of co-digestion of sewage sludge and organic wastes. *International Biodeterioration & Biodegradation*, **113**, 3-8.

Wirth, B., Reza, T., Mumme, J. 2015. Influence of digestion temperature and organic loading rate on the continuous anaerobic treatment of process liquor from hydrothermal carbonization of sewage sludge. *Bioresource Technology*, **198**, 215-222.

Xie, S., Hai, F.I., Zhan, X., Guo, W., Ngo, H.H., Price, W.E., Nghiem, L.D. 2016. Anaerobic co-digestion: A critical review of mathematical modelling for performance optimization. *Bioresource Technology*, **222**, 498-512.

Xie, S., Wickham, R., Nghiem, L.D. 2017. Synergistic effect from anaerobic co-digestion of sewage sludge and organic wastes. *International Biodeterioration & Biodegradation*, **116**, 191-197.

Xie, S., Wu, G., Lawlor, P.G., Frost, J.P., Zhan, X. 2012. Methane production from anaerobic co-digestion of the separated solid fraction of pig manure with dried grass silage. *Bioresource Technology*, **104**, 289-297.

Zhang, J., Lv, C., Tong, J., Liu, J., Liu, J., Yu, D., Wang, Y., Chen, M., Wei, Y. 2016. Optimization and microbial community analysis of anaerobic co-digestion of food waste and sewage sludge based on microwave pretreatment. *Bioresource Technology*, **200**, 253-261.

Zhang, L., Jahng, D. 2012. Long-term anaerobic digestion of food waste stabilized by trace elements. *Waste Management*, **32**(8), 1509-1515.

Zhang, R., El-Mashad, H.M., Hartman, K., Wang, F., Liu, G., Choate, C., Gamble, P. 2007. Characterization of food waste as feedstock for anaerobic digestion. *Bioresource Technology*, **98**(4), 929-935.