Kinetic parameter estimation model for anaerobic co-digestion of waste activated sludge and microalgae

Eunyoung Lee [a], Jewel Cumberbatch [b], Meng Wang [a], Qiong Zhang [a,*]

[a] Department of Civil and Environmental Engineering, University of South Florida, 4202 E Fowler Avenue, Tampa, FL 33620, United States

[b] Environmental Engineering Department, University of Florida, Gainesville, FL 32611, United States

*Corresponding author: Qiong Zhang, Tel: +1 813 974 6448; Fax: +1 813 974 2957; e-mail: qiongzhang@usf.edu

Abstract

Anaerobic co-digestion has a potential to improve biogas production, but limited kinetic information is available for co-digestion. This study introduced regression-based models to estimate the kinetic parameters for the co-digestion of microalgae and wasted activated sludge (WAS). The models were developed using the ratios of co-substrates and the kinetic parameters for the single substrate as indicators. The models were applied to the modified first-order kinetics and Monod model to determine the rate of hydrolysis and methanogenesis for the co-digestion. The results showed that the model using a hyperbola function was better for the estimation of the first-order kinetic coefficients, while the model using inverse tangent function closely estimated the Monod kinetic parameters. The models can be used for estimating kinetic parameters for not only microalgae-WAS co-digestion but also other substrates' co-digestion such as microalgae-swine manure and WAS-aquatic plants.

Keyword: Anaerobic co-digestion, Microalgae, Waste activated sludge, Kinetic parameter, First-order kinetic model, Monod model

1. Introduction

Anaerobic digestion technology has been used in waste management for several purposes such as waste stabilization, solids reduction, and energy production (Angelidaki et al., 2003; Kythreotou et al., 2014). With the increasing interest in protecting environments and producing renewable energy, this technology becomes more popular due to its ability to produce biogas from waste (Kythreotou et al.,

2014). However, anaerobic digestion of some substrates such as waste activated sludge, agricultural waste, and microalgae results in low biogas yield, because the substrate has low organic loadings (low carbon content) and high ammonia concentrations that negatively impact on the activity of methanogens during anaerobic digestion (Mata-Alvarez et al., 2014). Anaerobic co-digestion, which is the simultaneous digestion of two or more substrates, could be a feasible option not only to overcome this drawback by supplying missing nutrients from co-substrates and diluting the potential toxic substances, but also to stimulate synergistic effects on microorganisms (Mata-Alvarez et al., 2000). Many substrates, including animal waste, sewage sludge, municipal organic solid waste, agricultural waste, fats, oil, grease, and microalgae have been used for co-digestion (Mata-Alvarez et al., 2014). In particular, studies on anaerobic co-digestion using microalgae have been increased for the last decade because microalgae have an ability to treat wastewater with high biomass productivity (Pittman et al., 2011). Due to this ability, microalgae have been used for nutrient recovery in nutrient rich wastewater such as rejecting water from sludge dewatering (Pittman et al., 2011; Olsson et al., 2014). Moreover, wastewater treatment integrated with microalgae cultivation and subsequent production of biogas from the co-digestion using waste activated sludge (WAS) and microalgae can be one of the most promising options for renewable energy production at wastewater treatment plants (Ajeej et al., 2015; Wang et al., 2016).

Anaerobic co-digestion has the same mechanism as anaerobic digestion that consists of a series of biological conversion processes in which multiple microorganisms break down biodegradable organic substances, and these processes are described by four major steps, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Batstone et al., 2002; Gavala et al., 2003; Vavilin et al., 2008). It is generally accepted that hydrolysis and methanogenesis are rate limiting steps in the anaerobic digestion process (Gavala et al., 2003; Ariunbaatar et al., 2014). Due to enzymatic activity by hydrolytic bacteria to break down the large organic matters, hydrolysis is considered to be a slow reaction. On the other hand, methanogenesis is considered as another rate limiting step, because methanogenic bacteria require complex environmental conditions that are hard to maintain in digesters. For example, nitrogen contents

between 3.5 and 8.7% in the substrates may result in methanogenesis inhibition (Cotsta et al., 2012). When the pH drops below 7.0 as a result of fast acidogenesis and acetogenesis steps, the activity of the methanogens is inhibited (Schwede et al., 2013). For the co-digestion of microalgae and WAS, hydrolysis and methanogenesis can be also considered as the rate-limiting steps because microalgae affect these steps (Costal et al., 2012). For instance, a hemicellulose composition of the microalgae cell wall impacts on the hydrolysis of the co-digestion (Northcote et al. 1958). Also, a high ammonia concentration resulting from degradation of protein content in microalgae negatively affects the methanogenic bacteria activity (Mairet et al., 2011).

The rates of these two steps have been described by different kinetic models, such as the first-order kinetic model, Monod model, and Andrews model. (Kythreotou et al., 2014). Among these models, the first-order kinetic model was mostly used to explain the rate of hydrolysis, whereas the Monod model was commonly applied in kinetic modeling of methanogenesis. Vavilin et al. (2008) reviewed existing kinetic models for the hydrolysis of particulate organic materials in anaerobic digestion. For anaerobic digestion of complex organic substrate, they suggested a modified first-order kinetic model taking into consideration of non-biodegradable fraction of the substrate. In addition to improving the rate expression of the kinetic models, the determination of the kinetic parameters is critical for the overall model prediction.

The kinetic parameters are usually obtained from kinetic studies using an experimental approach (Lübken et al., 2015). This approach provides accurate kinetic information under specific conditions, but it requires time, energy, labor, and cost to obtain the results. There are many kinetic studies for anaerobic digestion, especially anaerobic digestion of sludge from wastewater treatment plant which has been well documented by Gavala et al. (2003). Based on the previous kinetic studies, it is found that majority of the studies focused on single substrates and limited studies dealt with determining the kinetic parameters for co-digestion. Costal et al. (2012) investigated methane production potential of anaerobic co-digestion of *Ulva* sp. and WAS in batch mode at mesophilic conditions. The parameters of the first-order kinetic

model for different ratios of co-substrates were determined in the study (Coastal et al., 2012). Neumann et al. (2015) studied anaerobic co-digestion of lipid-spent *Botryococcus braunii* with WAS and glycerol. They also determined the kinetic parameters for the first-order kinetic model under different ratios of the co-substrates. Zen et al. (2016) evaluated the technical feasibility of anaerobic co-digestion of mixed microalgae and food waste in batch tests and explained the kinetics of methane production using the first order kinetics. The results from these prior studies showed that kinetic parameter values were different between single and multiple substrates. Depending on a ratio of co-substrates on a volatile solid basis (or percentage), the kinetic parameters for the co-digestion can be quite different. In addition, the kinetic information for co-digestion of WAS and microalgae was very limited. Extensive experiments therefore need to be conducted in order to obtain kinetic parameters under different ratios of co-substrates.

This study aims at providing an alternative approach for estimating the kinetic parameters for co-digestion of microalgae and WAS under different ratios of co-substrates with limited kinetic experiments. The proposed kinetic parameter estimation models considered key factors which are ratios of co-substrates and the kinetic parameters for the single substrate. Among the existing kinetic models, the most applicable ones were selected - the modified first-order kinetic model for hydrolysis and the Monod model for methanogenesis (McCarty and Mosey, 1991; Vavilin et al., 2008). To demonstrate the applicability of the parameter estimation models, the models were applied to the published data from literature.

2. Methodology

2.1 Experimental method

2.1.1 Microalgae cultivation

Indigenous *Chlorella sp.* was cultivated in 2L batch glass photo-bioreactors in two times diluted real centrate. The enrichment and identification of the algal species was done as described in Halfhide et al. (2015). The centrate was collected from the Northeast Water Reclamation Facility, NWRF (located in

Clearwater, FL), which contains 397±145 mg NH_4^+-N/L and 238±59 mg TP/L. In order to remove particles, the centrate was filtered through glass fiber filters (Fisher Scientific, USA) with pore size of 0.45 μm. The detailed characteristics and preparation of the centrate were described in Lee and Zhang (2016). The reactors were maintained at 22±1°C in a temperature-controlled room. The cultures were kept suspended by aeration (0.03% CO_2). A 24-hour continuous light (about 9000 lux) was provided by 13W fluorescent lamps.

2.1.2 AD reactor set-up

Batch-type anaerobic digestion experiments were performed in duplicates of 100 mL glass serum bottles with a working volume of 40 mL for 20 days. The reactors were maintained at 35°C and manually mixed twice each day. Anaerobic digested sludge and waste activated sludge (WAS) were collected from NWRF. The anaerobic digested sludge was used as inoculum for the tests. The waste activated sludge was prepared by gravity setting or centrifugation, while the microalgae were harvested by centrifugation (3000 rpm, 15 minutes), in order to reach targeted volatile solids (VS) concentrations (5%). The characteristics of WAS, microalgae, and inoculum are shown in Table 1. To evaluate the effect of varying microalgae and WAS ratios on digestion performance, microalgae and WAS were added to the reactors to achieve the following mass (VS) composition: 100% WAS, 5% microalgae with 95% WAS, 10% microalgae with 90% WAS, 25% microalgae with 75% WAS, 40% microalgae with 60% WAS, 50% microalgae with 50% WAS, 75% microalgae with 25% WAS and 100% microalgae. A Substrate to Inoculum ratio (S/I) of 1 g VS/g VS was used for all experiments. Each bottle was purged with N_2 gas before sealing to remove oxygen.

2.1.3 Analytical methods

Total Chemical Oxygen Demand (COD), soluble COD, ammonium (NH_4^+-N), total solids (TS), VS, pH, biogas volume, methane content of the biogas were measured in this study. Total and soluble CODs were measured according to Standard Methods (5200B) using Orbeco-Hellige MR COD kits (Kit

number 2420711, testing Range 0-1500mg/L). Standard Methods were used for total solids (TS) and VS (Method 2540), pH (Method 2320B) measurements (APHA et al., 2012). Measurement of nitrogen in NH_4^+-N was adapted from a modified Willis et al. (1996) method by Kinyua (2013). Samples filtered through 0.45µm membrane filters (Fisherbrand™ General Filtration Membrane Filters, USA) were collected for soluble COD and NH_4^+-N analysis. Biogas volume was manually measured from the headspace of each digester by injecting a 50 mL glass syringe (Poulten & Graf Ltd., Germany) (Ashekuzzaman, and Poulsen, 2011; Wang et al., 2016). At each sample event, methane content in the biogas were measured through liquid displacement of CO_2 dissolved in alkaline solution (Ergüder et al., 2001).

2.2 Kinetic models applied

The concept of hydrolysis generally includes disintegration, solubilization and enzymatic hydrolysis as described in most of the literature (Vavilin et al., 2008; Batstone et al., 2002). The modified first-order kinetic model includes non-biodegradable fraction of the substrate, which are able to account for slow or non-degradable materials in the substrate (Vavilin et al., 2008). The adopted modified first-order kinetic model is described;

$$r_{hyd} = -k_{hyd} \cdot (S_P - \beta S_{P0}) \qquad (1)$$

where r_{hyd} is rate of hydrolysis, kg m^{-3} d^{-1}, S_p is particle substrate concentration, kg m^{-3}, S_{p0} is the initial particle substrate concentration, kg m^{-3}, k_{hyd} is the first-order rate coefficient, d^{-1}, and β is the non-biodegradable fraction of the substrate. In batch mode, the differential equation was written as follows:

$$\frac{dS_P}{dt} = r_{hyd} \qquad (2)$$

Methanogenesis is the most sensitive step for anaerobic digestion process and the rate is described by the Monod type model (Lawrence and McCarty, 1970; Pavlostathis et al., 1986);

$$\frac{dM}{dt} = r_m = \frac{k_m \cdot V}{K+V} \qquad (3)$$

where M is methane production, mL g^{-1} VS, r_m is rate of methane production, mL g^{-1}VS d^{-1}, K is half saturation coefficient, mg L^{-1}, V is volatile fatty acid (VFA) concentration, mg L^{-1}, k_m is the maximum substrate utilization rate, mL g^{-1} d^{-1}. In batch system such as Bio-Methane potential (BMP) assay, the ratio of S/I is an essential parameter that is able to affect the accumulation of VFA as well as the production of methane (Alzate et al., 2012). It is often observed that the digestion was inhibited by accumulation of VFA at a high S/I ratio (Alzate et al., 2012; Zhao et al., 2014). Zhao et al. (2014) reported that there was no sign of VFA inhibition for anaerobic digestion of microalgae at the S/I ratio ≤ 1.0. According to Costal et al. (2012) who studied the co-digestion of WAS and microalgae with S/I ratio of 2.8, it was reported that there was no inhibition of methanogenesis from accumulation of VFA, because its concentrations were below 50 mg L^{-1}. Thus, since the S/I ratio of this study was below 2.8, inhibition of methanogenesis was therefore not considered. In anaerobic digestion, methane production is proportional to produced VFA (Rahman et al., 2013; Kamalak et al, 2002) (Equation 4).

$$M_t - M = \alpha \cdot V \qquad (4)$$

where α is conversion coefficients ($\alpha=M_t/V_t$), V_t is a total VFA concentration, mg L^{-1}, V is volatile fatty acid (VFA) concentration, mg L^{-1}, M is the accumulated methane production (CH_4) at 35°C at the time t, mL g^{-1}VS, and M_t is the total methane production for 20 days at 35°C, mL g^{-1}VS. Substituting Equation 4 to Equation 3 resulted in the final kinetic expression as listed in Table 2. The parameters, including k_{hyd}, β, k_m, and K', were determined by fitting the integrated forms (Table 2) to experimental data using minimization of an Objective Function (OF, Equation 5)

$$OF = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(C_M - C_P)^2} \qquad (5)$$

where n is number of data points, C_M is particulate COD concentrations or the methane productions from experiments, and C_P is the predicted particulate COD concentrations or the methane productions from the model. This determination was achieved by using a Generalized Reduced Gradient (GRG) Nonlinear

solver tool in Microsoft Excel. R^2, which is a common method to evaluate the model fit, was calculated and provided.

2.3 Development of the kinetic parameter estimation models

Anaerobic digestion kinetics are generally affected by several factors such as temperature, pH, types of substrates, mixing, and S/I ratio (Manea et al., 2012). The factors of temperature, pH, mixing, and S/I ratio are relatively constant for anaerobic digestion at mesophilic domain: Temperature is generally kept in 35-37°C; Mixing is usually applied to provide homogenized conditions for the digestion; pH is kept at a neutral condition; and S/I ratio is usually applied in the range from 0.5 to 3. Since anaerobic co-digestion keeps these factors constant, co-substrate types and ratios are considered as major factors in the co-digestion kinetic modeling. Previous studies showed that the kinetic parameter values varied according to the type of substrates (Gavala et al., 2003; Vavilin et al., 2008) and the ratios of co-substrate (Costal et al., 2012; Neumann et al., 2015; Zen et al., 2016). In prior studies, most of the kinetic parameter values for the co-digestion were higher than the parameters for one of the single substrates. In addition, the maximum kinetic parameter value was found at the certain ratio of co-substrates. For example, the k_{hyd} values increased for the co-digestion of microalgae and WAS when the ratios of microalgae increased to the threshold point (this point refers to the best combination for anaerobic co-digestion of microalgae and WAS), and then gradually decreased to the value for the single substrate. Thus, types and ratios of co-substrates were selected as indicators in the parameter estimation model. In the model, kinetic parameters for a single substrate were used as a substitute for substrate types because they are directly related to the types of substrate.

For model framework, hyperbolic and inverse tangent relationships were adopted in order to explain the trend of kinetic parameter for co-digestion, which are shown in Equations 6 and 7. In general, the hyperbolic and inverse tangent-based equations are able to provide the S-shaped curve. These functions are often used in modeling of biological systems. For example, the hyperbolic function was applied to describe growth kinetics and enzyme kinetics, while the inverse tangent function was used in

soil respiration (Adair et al., 2008; Del Grosso et al., 2005; Panikov, 1995). At a transition point of the curve, the inverse tangent function has slightly steeper slope representing the greater rate of change compared with hyperbolic function.

In the models, the constant *a* is introduced to avoid infinite value in denominator as well as to explain the synergetic effect of the co-substrates. For example, when constant *a* decreases, the K_E value increases. In other words, the lower *a* value indicates that substrates have the higher synergetic effect in co-digestion.

$$K_E = \frac{K_A * P_A}{P_A + a} + (K_{WAS} \cdot P_{WAS}) \qquad (6)$$

$$K_E = K_A \cdot \mathrm{ATAN}\left(\frac{P_A}{a}\right) + (K_{WAS} \cdot P_{WAS}) \qquad (7)$$

where K_E is the estimated kinetic parameters, which could be the first-order rate coefficient or the maximum substrate utilization coefficient for co-digestion of WAS and microalgae. K_{WAS} and K_A are the first-order rate coefficients or the maximum substrate utilization coefficient for anaerobic digestion of waste activated sludge only and anaerobic digestion of microalgae only, respectively, P_A and P_{WAS} are the percentage of microalgae and waste activated sludge by mass of volatile solid, respectively.

Constant *a* was calibrated by minimizing the total relative error between the parameters determined from experiments and the parameters estimated from the models. In order to evaluate the performance of the estimation models, Akaike Information Criterion (AIC) was used as a statistical index (Motulsky and Christopoulos, 2004).

$$AIC = n\ln\left(\frac{RSS}{n}\right) + 2(N+1) + \frac{2(N+1)(N+2)}{(n-N-2)} \qquad (8)$$

where RSS is residual sum of squares, n is number of data points, and N is number of model parameters. The highest quality of the model will result in the smallest AIC. Also, R^2 and normalized root mean

squared error (NRMSE) were calculated to provide additional information about the goodness of fit for the models. The NRMSE was calculated based on following relationship;

$$NRMSE = \frac{\sqrt{\sum_{n=1}^{n}\frac{(P-C)^2}{n}}}{h} \times 100 \qquad (9)$$

where P is predicted kinetic parameters using the models, C is kinetic parameters determined from the experimental data in this study or the published studies, and h is the mean of the kinetic parameters C. If the model accurately predicts the kinetic parameter (e.g., k_{hyd} or k_m), R^2 should be close to 1, and AIC tends to be low. According to Jamieson et al. (1991), a model simulation was considered to be acceptable when the NRMSE is less than 30%. Based on the statistical indices, better models for hydrolysis and methanogenesis were selected using the experimental results from this study. The models were then tested using the data from published studies and the NRMSE criteria (NRMSE<30%) was used in this case to determine the acceptability of the model.

3. Results and Discussions

3.1 Kinetic parameters for hydrolysis and methanogenesis

The modified first-order kinetic model and Monod type kinetic model were applied for hydrolysis and methanogensis in the co-digestion of microalgae and WAS, respectively. Figure 1 shows simulated and experimental results for hydrolysis. The simulated data showed a relatively good fit to experimental data for Particulate Chemical Oxygen Demand (PCOD) with $R^2 > 0.8$. The R^2 of the simulated result for 100% waste activated sludge was 0.68, which was lower than the values for other co-substrate ratios. Due to the uneven particle size as well as amount of particles in the samples, the change of PCOD value for 100% WAS were not noticeable during days 0-3. Thus, this affected the goodness of fit of the model. Based on the results, the modified model was able to explain the hydrolysis of microalgae and WAS during the co-digestion. Shimizu et al. (1993) and Morand and Briand (1999) also applied the first-order kinetic model considering non-degradable portions for anaerobic digestion of WAS and microalgae (*Ulva*

sp.), respectively. They also concluded that the modified first-order kinetic model was able to explain the hydrolysis of these substrates in their studies.

Simulated results using the Monod model and experimental results of methane accumulation for different ratios of microalgae and WAS are shown in Figure 2. The Monod model slightly overestimated accumulated methane during days 10-18, but the production trend was well described with $R^2 > 0.9$ for all compositions. Siegrist et al (2002) also reported that the Monod type kinetic model was able to describe the conversion of VFA from wasted sewage sludge to methane in anaerobic digestion.

The hydrolysis and methanogensis kinetic parameters obtained from the models are presented in Table 3. Based on the kinetic parameter values for hydrolysis obtained from experimental data, the values of the kinetic parameters were increased, as the microalgae proportion increased from 0 to 10%. This result indicates that the co-digestion can improve the reaction rate of the hydrolysis. It was observed that the composition of 10% microalgae with 90% WAS has the highest k_{hyd} value (0.14) and the k_{hyd} for the composition of 75% WAS with 25% microalgae was the second highest (0.13). These results indicate that the co-digestions of microalgae and WAS at these ratios were able to achieve the synergetic effect on the substrate biodegradability. However, the values were gradually decreased, as the microalgae increased from 25% to 100% in the substrates. It is because increasing microalgae that have complex cell walls slows down hydrolysis (Frigon et al., 2013; Wang et al., 2013). Costal et al. (2012) reported that the first-order kinetic model successfully described the hydrolysis for the co-digestion of microalgae and WAS. They observed that the highest value of k_{hyd} was obtained in the co-digestion of 15% VS microalgae with 85% VS mixed waste sludge (primary and secondary waste sludge), and concluded that this composition would have the best synergetic effect for the co-digestion. Their study also observed the similar trend that the k_{hyd} value reached to the maximum value and then decreased as percentage of VS microalgae increased.

On the other hand, it was observed that the kinetic parameter values for methanogenesis obtained from experimental data increased with increasing microalgae ratio in the substrates. This is because the

cell contents of microalgae (high lipid contents) may improve methanogen activity and increase the rate of methanogenesis. Based on the Table 3, 100% microalgae resulted in the highest k_m value (32.8), and the k_m for the composition of 60% WAS with 40% microalgae was the second highest (32.3), but these two values were comparable.

3.2 Estimation of kinetic parameters by the proposed models

Based on the kinetic parameters for the single substrates and ratio of microalgae in co-substrate, the k_{hyd} and k_m for different compositions of WAS and microalgae were estimated using the kinetic parameter estimation models (Equations 6 and 7). Figure 3 shows the results for estimated parameters from the models and kinetic parameters obtained from experiments as listed in Table 3. The constants in Equations (6) and (7) for hydrolysis were 0.10 and 0.38 respectively, while the constants in Equations (6) and (7) for methanogenesis were 0.21 and 0.55, respectively. Based on the results, both models were able to estimate the kinetic parameters for the first-order kinetic model and Monod model. In order to find the better model to estimate the kinetic parameters for the co-digestion, R^2, AIC, and NRMSE for hydrolysis and methanogenation were established as listed in Table 4.

In Figure 3(a), it was shown that the two proposed models were both able to capture the trend of hydrolysis that was discussed in the section 3.1. However, Equation (6) with hyperbolic relationship more closely estimated the kinetic parameters of hydrolysis between 0-50% microalgae in the substrates. When comparing the two models, Equation (6) was the better estimation model for hydrolysis, which has the higher R^2, lower AIC, and lower NRMSE than equation (7). On the other hand, Figure 3(b) showed that the inverse tangent relationship more closely estimated k_m values for lower microalgae ratio in the substrates (below 30%). Thus, Equation (7) was better for estimating k_m values for methanogenesis which has the higher R^2, lower AIC, and lower NRMSE than equation (6).

The kinetic parameter estimation models were also tested using published data for the co-digestion. Equation (6) was applied for estimating k_{hyd}, while equation (7) was used for estimating k_m. The results for estimating k_{hyd} are shown in Figure 4. Figure 4(a) presents the results for the kinetic parameters

obtained from Costal et al. (2012) and the parameters estimated from the model. The model was able to estimate k_{hyd} values with a NRMSE of 29% with the several values underestimated. This might be due to properties of the sludge used in Costal et al. (2012). In their study, the mixture of primary sludge and waste activated sludge was used, and this mixture might affect the hydrolysis kinetics.

Figure 4(b) shows kinetic parameters from Neumann et al. (2015) and estimated kinetic parameters using the proposed model. The data for the kinetic parameters from their study have a distinctive shape, and this is different to the results shown in Figure 3(a). In their results, the values of the kinetic parameters were increased, as the microalgae increased from 0% to 75%. This is because they used lipid-spent microalgae that have already broken down microalgae cell walls from lipid extraction, and the cell disruption resulted in the increase in the hydrolysis rate (Ramos-Suárez and Carreras, 2014). In this condition, the kinetic parameter value for microalgae was higher than that of the WAS (Typically, the kinetic parameter value for microalgae is lower than that of WAS), and this affected the trend of the kinetic parameter for co-digestion as a function of substrate ratio. For this case, the proposed model slightly overestimated the k_{hyd} values at 25% lipid-spent *Botryococcus braunii* with 75% WAS and slightly underestimated the k_{hyd} values at 75% lipid-spent *Botryococcus braunii* with 25% WAS. Although the model did not provide the good curve trend, the model's NRMSE was 27%, which is considered to be within the acceptable range for model prediction.

Astals et al. (2015) investigated anaerobic co-digestion of pig manure and algae (*Scenedesmus sp.*) under mesophilic condition. They assessed kinetics and substrate biodegradability for hydrolysis using the first-order kinetic model. Figure 4(c) shows the results for the kinetic parameters obtained from experiments and estimated from the proposed model. The model underestimated the kinetic parameters at compositions of 30% and 50% microalgae, due to the constant *a* in the model. The proposed model was developed to estimate the kinetic parameter values for microalgae and WAS, and the constant *a* reflected the synergetic effect of the microalgae and WAS. However, in Astals et al. study the pig manure was used as one of co-substrates, which has different characteristic to WAS. Although the different co-substrates were used, the model was still able to determine the kinetic parameters with the NRMSE of 24%.

Zen et al. (2015) applied *Egeria densa* which is an aquatic plant, and they evaluated the feasibility of anaerobic co-digestion of *Egeria densa* and WAS with different VS ratios. It was observed that the model slightly underestimated k_{hyd} values due to the same reason as discussed above. Although the estimated kinetic values were lower than the values obtained from experiment results, the model was able to estimate the kinetic parameters with a low NRMSE (14%). In general, Equation (6) was able to estimate the k_{hyd} value within acceptable range.

Figure 5 shows the comparison of k_m values predicted using Equation (7) and obtained from published data by Wang et al. (2013), Gordon (2015), Kim and Kang (2015), and Lu and Zhang (2016). Gordon (2015) investigated the co-digestion of microalgae and WAS as a method to recover and recycle nutrients in algal biofuel systems. They conducted the co-digestion of microalgae (*Chlorella sp.*) and WAS by varying the ratio of microalgae to WAS in the substrates. The kinetic parameters for the Monod model were obtained from their experimental results for average cumulative methane production, which is shown in Table S1. It was found that the Monod model fit their experimental data well with R^2>0.84. As shown in Figure 5(a), estimated k_m from the model was close to k_m values obtained from experimental results (NRMSE=17%). For 25% microalgae in the substrates, the kinetic parameter value obtained from experimental data was higher than the predicted kinetic parameter value.

Lu and Zhang (2016) evaluated the feasibility of using septic sludge and microalgae (*Chlorella sp.*) for anaerobic co-digestion. Based on their biogas data, the kinetic parameters were determined, which is shown in Table S2. Figure 5(b) shows the k_m values with different microalgae and septic sludge compositions. The model estimations were very close to k_m values obtained from experimental data (NRMSE<7%) and the model was able to capture the distinctive curve trend that was different to the results shown in Figure 3(b); the values were decreasing with an increase of microalgae.

Wang et al. (2013) investigated anaerobic co-digestion of microalgae (*Chlorella sp.* and *Micractinium sp*) and WAS. The Monod model well described the production of biogas for the co-digestion, and the kinetic parameters are presented in Table S3. In Figure 5(c), the estimated k_m from the

model and the k_m calculated from experimental data were compared. It was found that the k values were slightly underestimated, but the model was able to estimate k_m with a NRMSE of less than 10%.

Kim and Kang (2015) evaluated methane production by the anaerobic co-digestion of different mixtures of food waste leachate, microalgal biomass (*Chlorella sp.*), and raw sewage sludge. Methane production data for the co-digestion of microalgae and raw sewage sludge were taken from their study, and the methane production kinetics were described via fitting their experimental data to the Monod model. The kinetic parameters were shown in Table S4. The k_m values of their study were lower than other studies because of low initial volatile solid concentrations for the co-substrate. Figure 5(d) compared the k_m values predicted from the proposed model and estimated from experimental data. The model closely estimated the k_m values for the co-digestion with a NRMSE of 6%.

Based on the results presented above, the proposed models can generally predict the kinetic parameters in the first-order kinetic for hydrolysis and the Monod model for methanogenesis in co-digestion of microalgae and WAS as well as other combinations with microalgae within the acceptable range. The observed fluctuations in the estimates might be related to two factors; original kinetic parameter values for single substrates, and the constant *a* in the model.

The kinetic parameter values for single substrate were one of the important factors in the kinetic parameter estimation model. Thus, it is important to obtain accurate kinetic parameter values for single substrates from the experiments in order to improve the model prediction. Another important factor that affected the model estimation was the constant *a*. In this study, the model was developed to estimate the kinetic parameters for the co-digestion of microalgae and WAS. Thus, the model component, particularly the constant *a*, was used to explain the synergetic effect of these two substrates. When the model applied for the other substrates, it was observed that the model predictions were underestimated or overestimated, because the synergetic effect will be different for different substrates. In order to apply for other co-digestion cases, the constant *a* also needs to be determined based on substrates characteristics, such as

particle size and C/N ratios. Therefore, future research may focus on these limitations to improve the applicability of the model to various substrates for the co-digestion.

4. Conclusions

In order to estimate the kinetic parameters for anaerobic co-digestion of microalgae and WAS, two estimation models were proposed based on the kinetic values for single substrates and the ratios of the co-substrates. It was observed that the model using a hyperbola function was better for the estimation of the first-order kinetic coefficient, whereas the model using inverse tangent function closely estimated the Monod kinetic parameters. When the models were applied to other cases in the published studies, they were able to estimate kinetic parameters in those studies within an acceptable range even under different conditions from this study.

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Figure Caption

Figure 1. Simulated and experimental data for Particulate Chemical Oxygen Demand (PCOD) with different compositions of Volatile Solids (VS) (○ and ●: experimental data; solid and dashed line: simulated results using the modified first-order kinetic model); (a) 100% Wasted Activated Sludge (WAS) and 5% microalgae (A) with 95% WAS, (b) 10% A with 90% WAS and 25% A with 75% WAS, (c) 40% A with 60% WAS and 50% A with 50% WAS, and (d) 75 % A with 25% WAS and 100% A

Figure 2. Simulated and experimental data for cumulative methane with different compositions of Volatile Solids (VS) (○ and Δ: experimental data; solid and dashed line: simulated results using the Monod model); (a) 100% Waste Activated Sludge (WAS) and 5% microalgae (A) with 95% WAS, (b) 10% A with 90% WAS and 25% A with 75% WAS, (c) 40% A with 60% WAS and 50% A with 50% WAS, and (d) 75 % A with 25% WAS and 100% A

Figure 3. Comparisons for simulated data from two models; (a) hydrolysis; (b) methanogenesis

Figure 4. Comparisons between k_{hyd} predicted from the proposed model and k_{hyd} estimated from literature data; (a) Costal et al. (2012); (b) Neumann et al. (2015); (c) Astals et al. (2015); and (d) Zen et al. (2015)

Figure 5. Comparisons between k_m predicted from the proposed model and k_m estimated from literature data: (a) Gordon (2015); (b) Lu and Zhang (2016); (c) Wang et al., (2013); and (d) Kim and Kang (2015)

Figure 1

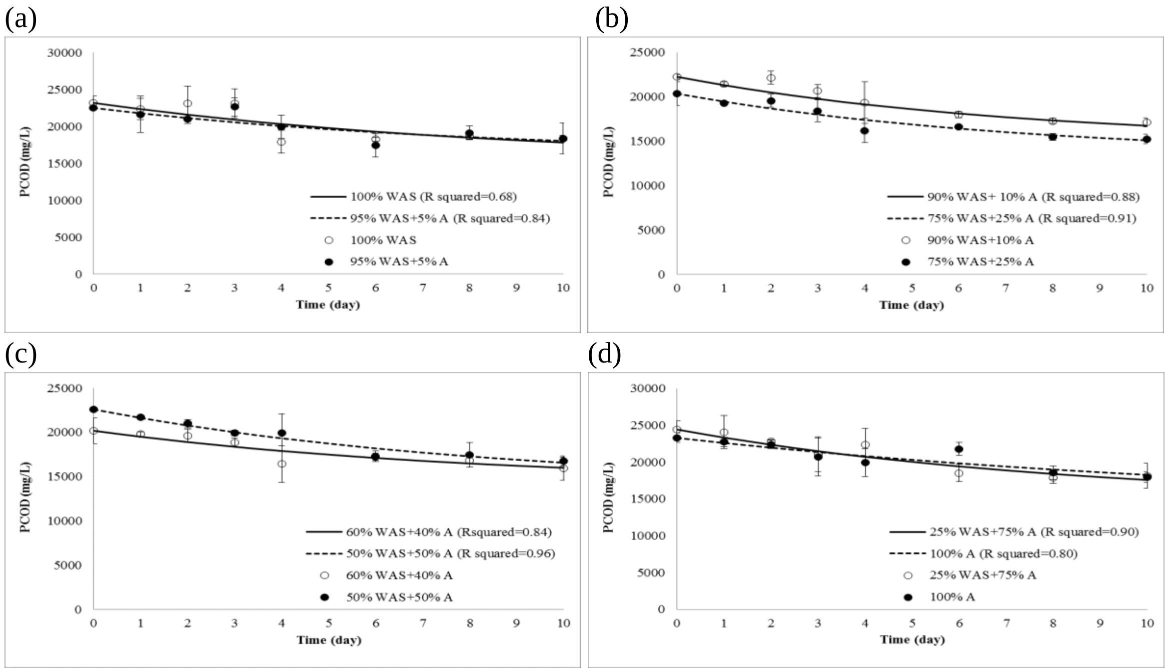

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Figure 2

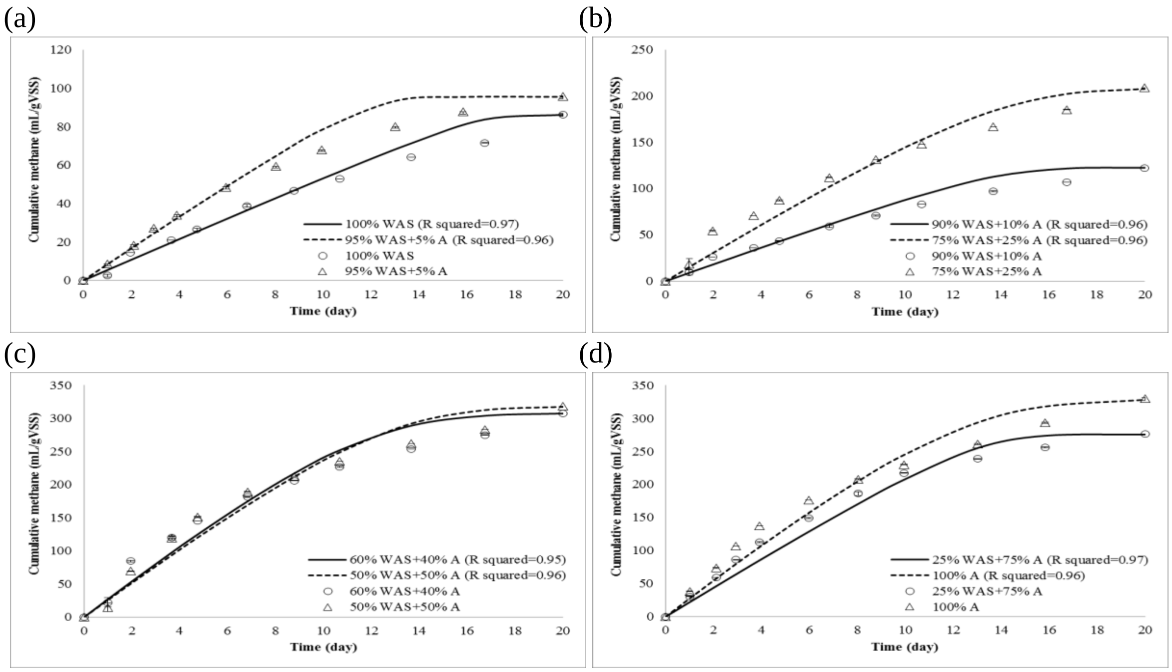

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Figure 3

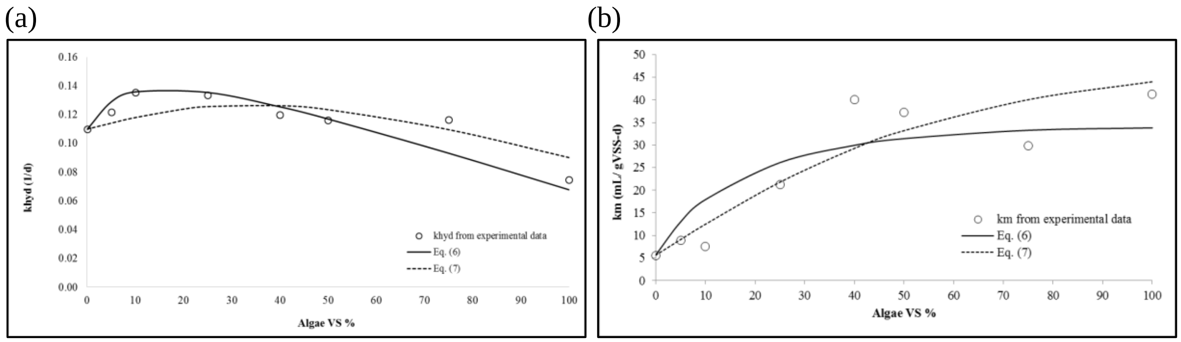

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Figure 4

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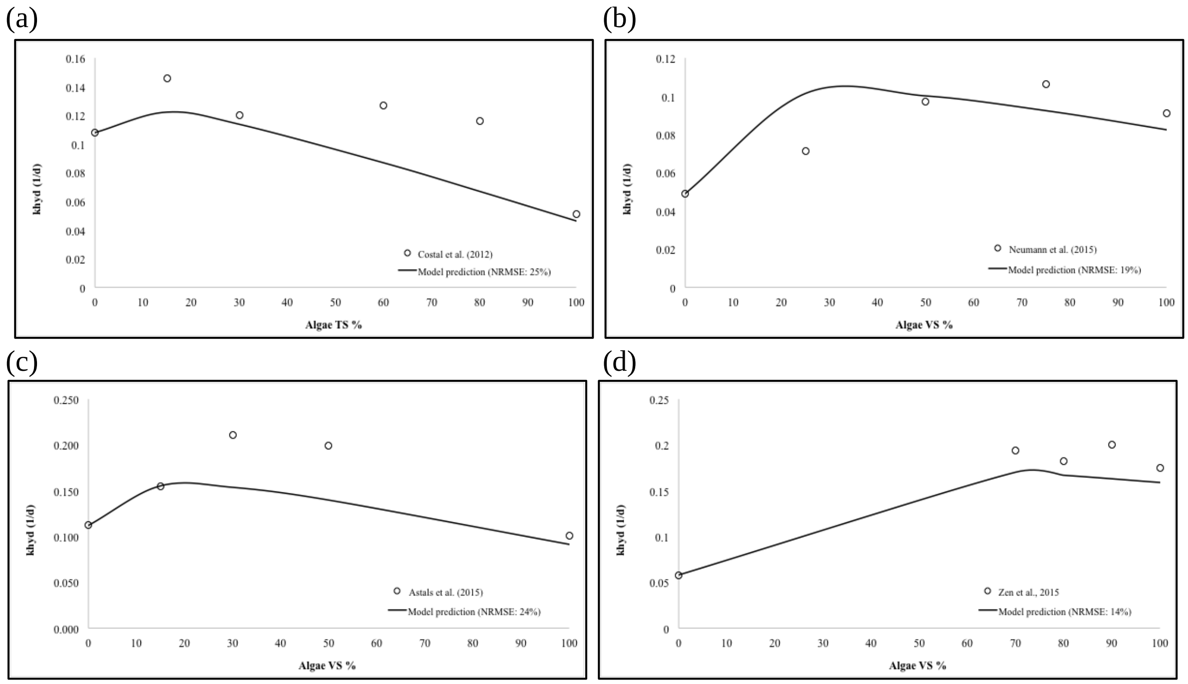

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Figure 5

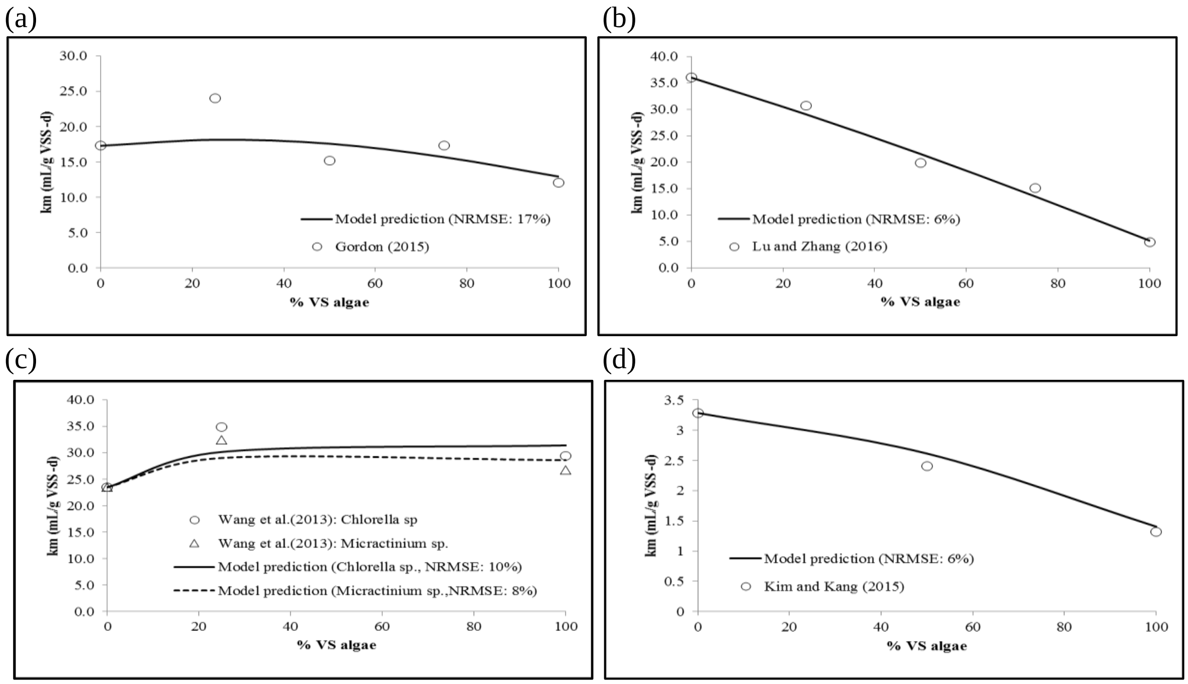

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Table 1. Characteristics of waste activated sludge, microalgae, and inoculum

Parameters	Microalgae	Waste activated sludge	Anaerobic inoculum
TS (g/L)	76.5±3	21.1±1.2	26.7±4.5
VS (g/L)	48.7±1.8	15.2±0.8	18.8±3
COD (g/L)	73.8±0.2	20.9±0.6	11.4±0.9
TN (mg/L)	1120±57	1590±74	739±20
TP (mg/L)	136±13	272±19	562±18

Table 2. Kinetic models and integrated equations for anaerobic digestion

Steps	**Models**	**Kinetic models**	**Integrated forms**
Hydrolysis	Modified 1st order model	$r_{hyd} = -k_{hyd} \cdot (S_P - \beta S_{P0})$	$S_P = (1-\beta)S_{P0}e^{-k_{hyd}t} + \beta S_{P0}$
Methanogenesis	Monod type model	$r_m = \dfrac{k_m \cdot \frac{M_t - M}{M_t}}{K' + \frac{M_t - M}{M_t}}$ *$K'=K/V_t$	$K' \ln\left(1 - \frac{M}{M_t}\right) - \frac{M}{M_t} + \frac{k_m}{M_t} t = 0$

Table 3. Kinetic parameters for hydrolysis and methanogenesis

S/I ratio (gVS/gVS)	Substrate (% by VS)		Modified 1st order equation			Monod type model		
	WAS	A	k_{hyd} (1/d)	β	R^2	k_m (mL/gVSS-d)	K'	R^2
1	100	0	0.11	0.65	0.68	5.56	0.03	0.97
1	95	5	0.12	0.72	0.84	8.89	0.06	0.96
1	90	10	0.14	0.67	0.88	9.81	0.07	0.96
1	75	25	0.13	0.65	0.91	17.4	0.12	0.96
1	60	40	0.12	0.70	0.84	32.3	0.18	0.95
1	50	50	0.12	0.61	0.96	30.0	0.15	0.96
1	25	75	0.12	0.60	0.90	23.7	0.07	0.97
1	0	100	0.07	0.59	0.80	32.8	0.18	0.96

* S/I ratio: substrate to inoculum ratio; VS: volatile solids; WAS: waste activated sludge; A: microalgae

Table 4. Goodness of fit for two kinetic parameter estimation models

Items	**Hydrolysis**		**Methanogenesis**	
	Equation (6)	**Equation (7)**	**Equation (6)**	**Equation (7)**
R^2	0.73	0.67	0.80	0.82
AIC	-70.4	-68.9	29.4	28.5
NRMSE	2.8%	3.1%	9.8%	8.6%