

Kinetic of Biogas Production in Co-Digestion of Vegetable Waste, Horse Dung, and Sludge by Batch Reactors

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Abstract. Batch experiments were performed firstly to evaluate co-digestion of vegetable waste (VW), horse dung (HD), and sludge (S). All reactors were set at a temperature of 37°C, pH of 6.7, and total solid 2.5%. Each single-substrate in the mixture played a significant role. In which, VW contributed mainly to the formation of biogas yield, S and HD played nutrient balance role. The biogas yield was in the range of 168-554 Nml/g-TS. Especially, the biogas yield could be estimated from the proportion of the substrates by equation $G1_{Nml/g-TS} = 53.7 + 7.448 \times VW(\%) + 1.922 \times HD(\%)$ or from nutrient ratio (C/N) by equation $G2_{Nml/g-TS} = 1341 - 48.46 \times C/N$. Further, the experimental data was applied to evaluate the kinetic equations of biogas production including the Gompertz (G) and Logistic (L) models. Constants in both models were found out by using the least squares fitting method. Both models showed high potential, in which, G model was completely better than L model. However, both models failed at time $t=0$ day. Moreover, the constant λ in models did not reflect the right definition itself, it was merely a mathematical constant.

1. Introduction

Anaerobic digestion (AD) is defined as a series of processes including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In which, microorganisms degrade down biodegradable materials in the condition of the oxygen absence [1]. Generation of solid waste containing a large amount of bio-waste increases rapidly in the recent years causing numerous problems (such as human health, the environmental pollution, the economic burden, ..etc.) that humankind has to deal. Hence, the AD of organic waste materials has been attracted remarkable attention within the scientific community for many years because of bringing two benefits include treating waste and producing biogas as an alternative energy source [2-4].

Inhibition of AD may occur because of losing the nutrient balance including excessiveness of macronutrients (Na, K, etc.) or deficiency of trace elements (Zn, Fe, Mo, etc.) [5, 6]. Particularly, the nutrient balance can be relatively measured by based on C/N ratio. The C/N ratio in the feedstock is too high pointing that the feedstock is not sufficient of nitrogen which needs for the build-up of microbial mass. Low C/N ratio leads to high concentration of generated ammonia, which is harmful to the AD processes [7]. Thus, the combination of the low nutrient material and the high nutrient material is the great ideal to reach nutrient balance. Dai, et al. [8] reported that co-digestion of grass and waste activated sludge enhanced gas production and methane content. Riggio, et al. [9] used a mixture of cow slurry, olive pomace, and apple pulp for the AD, and reported that stable gas generation was obtained with a mixture containing 85% cow slurry, 10% olive pomace, and 5% apple pulp. Wang, et al. [10] performed co-digestion of dairy manure, chicken manure, and wheat straw with the conclusion that co-digestion was better than individual digestion for methane potential. In fact, co-digestion has



been studied so much for recent years. However, the combination of vegetable waste (VW), sludge (S), and horse dung (HD) has been still left open-questions.

The final goal in the field of the AD aims to reach high biogas yield. Hence, the accumulation of biogas production is considered as the most important. This process can be described by the biogas production kinetic models. Biogas is generated under the activities of anaerobic microorganism hence many studies used growth kinetics to describe the biogas production [2, 11]. Growth curve often displays a phase in which the specific growth rate begins at a value of zero in a certain period (resulting in a lag time $-\lambda$) and then increases to a maximal value (μ_m). Additionally, growth curve contains a final phase in which the growth rate reduces and finally reaches zero so that an asymptote (A) is reached [12]. In which, the modified Gompertz (G) model and logistic (L) model have been received the most attention by good simulation [11, 13, 14]. However, the comparison between the G model and the L model for simulation of cumulative gas production has been not taken care of properly.

This study uses vegetable waste, sludge, and horse dung as feedstock for co-digestion firstly to evaluate different proportions of the mixture. Further, the influence of the C/N ratio to biogas potential is also investigated to contribute information about optimal C/N ratio. The obtained data from co-digestion is used for G and L models to simulate accumulative gas production. In which, advantages and disadvantages of each model are discussed to have an overview in applying the gas production kinetic models.

2. Materials and methods

2.1. Preparation of substrates

Vegetable waste (VW) and horse dung (HD) were collected in Jul 2017 at Okayama University. Raw materials were cut by a household grinder. Sludge (S) was collected from the digestion plant in Kobe City. All materials were stored in a refrigerator below 4°C until use.

2.2. Analytical methods

The 2400 series II CHNS/O analyzer (PerkinElmer) was used to analyze C/N ratio in the raw materials. Total solid (TS) was measured by the standard method 1684-EPA (2001); pH value was determined as following APHA (2000) SM 4500-H+ method by using Laqua-twin pH meter (Horiba).

Gas volume was measured by using the gas bag and a 120ml syringe. The temperature of the gas bag was observed every day for transferring gas volume into standard condition (25°C; P = 1at).

2.3. Reactors

By changing proportion of vegetable waste, horse dung, and sludge in the mixture, there were seven reactors (type 500ml) with different C/N ratios as shown in Table 1. All reactors were set up with a temperature of 37°C, TS of 2.5%, and initial pH of 6.7.

Table 1. Characteristics of the substrate inside reactors

No.	R1	R2	R3	R4	R5	R6	R7
Vegetable (%)	0	50	50	33.3	66.6	16.7	16.7
Horse dung (%)	100	50	0	33.3	16.7	66.6	16.7
Sludge (%)	0	0	50	33.3	16.7	16.7	66.6
C/N	22.5	16.8	18.7	19.8	16.0	21.1	23.9

2.4. Gas production kinetic models

The original Gompertz function is written as:

$$G_t = A \times \exp[-\exp(b - ct)] \quad (1)$$

Where, G_t , accumulation gas production (ml/g-TS);

A, biogas production potential (ml/g-TS);

b, c: constants of model;

t, cumulative time for biogas production (days).

At the flection point, where $t=t_o$, the second derivative is equal to zero, and the first derivative reaches the maximum value (μ_m -ml/g-TS)

$$\left\{ \begin{aligned} \left(\frac{dG_t}{dt} \right)_{t_o} &= A \cdot c \cdot \exp[-\exp(b - c \cdot t_o)] \cdot \exp(b - c \cdot t_o) = \lambda_m & (2) \\ \left(\frac{d^2G_t}{dt^2} \right)_{t_o} &= A \cdot c^2 \cdot \exp[-\exp(b - c \cdot t_o)] \cdot \exp(b - c \cdot t_o) \cdot [\exp(b - c \cdot t_o) - 1] = 0 & (3) \end{aligned} \right.$$

Moreover, the lag time (λ -day) is defined as the t-axis intercept of the tangent through the flection point, then:

$$0 = (\lambda - t_o) \cdot \left(\frac{dG_t}{dt} \right)_{t_o} + G_{t=t_o} \quad (4)$$

By solving the equations of (2), (3), and (4): $c=\mu_m \cdot e/A$; $b=(\mu_m \cdot \lambda \cdot e+A)/A$. Thus, the equation (1) can be rewritten as (called the modified Gompertz model):

$$G_t = A \times \exp \left\{ -\exp \left[\frac{\mu_m \cdot e}{A} (\lambda - t) + 1 \right] \right\} \quad (5)$$

By the same, the original logistic function $L_t = A \times [1 + \exp(b - ct)]^{-1}$ can be rewritten as:

$$L_t = A \times \left\{ 1 + \exp \left[\frac{4 \cdot \mu_m}{A} (\lambda - t) + 2 \right] \right\}^{-1} \quad (6)$$

3. Results and discussions

3.1. Biogas production

Experimental data on biogas yield was shown in **Figure 1**. In which, the whole digestion process had completed for approximately 45 days. Biogas accumulation was obtained in the range of 168-554 Nml/g-TS with the order as following $R7 < R1 < R6 < R4 < R3 < R2 < R5$. The biogas yield in this study could be compared with others studies about co-digestion. For instance, Heo, et al. [15] performed co-digestion of food waste (FW) and waste activated sludge, reported that biogas potential was from 170 to 443 ml/g-TS. Dong, et al. [16] studied co-digestion of municipal solid waste and obtained biogas yield of 260, 263, 294 ml/g-TS respectively at different total solids of 16%, 13.5%, and 11%. Zhang, et al. [17] focused on co-digestion of FW and cattle manure (CM), reported that biogas production of 484, 441, and 448 ml/g-TS were corresponding to FW/CM of 2, 3, and 4 respectively.

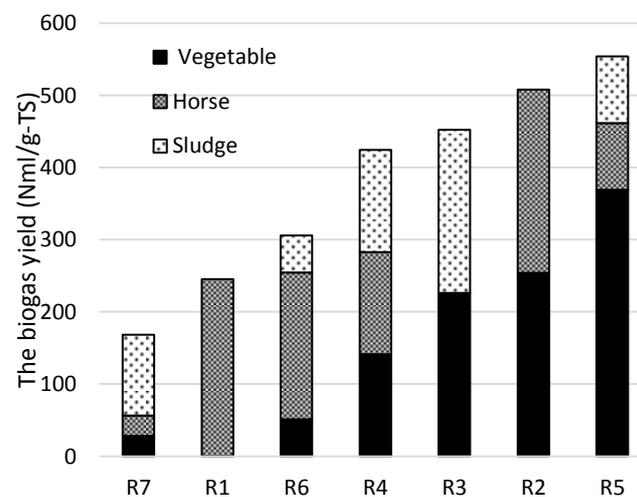


Figure 1. Biogas yield in reactors.

The lowest values of both the biogas yield and the biogas production rate were found in reactor R7 (168 Nml/g-TS) which contained 66.6% sludge (C/N=29.7). This result was derived from the high C/N ratio leading to deficiency of nitrogen in the feedstock, dragged on the inhibition as reported by Hartmann and Ahring [7]. Except for R7, the biogas yield from co-digestion was significantly higher than that of mono-digestion of horse dung (see Figure 1). The similar results were reported by Smith, et al. [18] and Kalia, et al. [19]. This reinforced the conclusion that co-digestion reduces inhibition and improves biogas production. The second lowest biogas production was in reactor R1 with 100% HD in the feedstock. This result can be explained by HD contains a large amount of cellulose which has been recommended not suitable for the AD [20, 21].

Moreover, except for R7, biogas yield increased accompany with lifting the proportion of VW (see Figure 1). And the highest biogas yield (554 Nml/g-TS) was found in reactor R5 that contained a large amount of VW (66.6%). Hence, a mixture with the proportion of a small amount of sludge, a little amount of HD, and a large amount of VW is recommended for the aim of high biogas yield. In addition, many studies reported that too low C/N in feedstock leads to increase ammonia formation which is toxic to the AD [3, 7]. Thus, a too high proportion of VW (C/N=13.2) leads to occur inhibition instead of enhancing biogas production. Overall, each raw material in this study evenly played a significant role in balancing nutrient of AD process.

Cumulative gas production could be predicted based on the substrate proportion by using the linear regression as equation $G1 = 53.7 + 7.448 \times VW(\%) + 1.922 \times HD(\%)$ (7). **Table 2** showed the significance of all the regression coefficients for equation (7). This result apparently quantized the role of each single-substrate in biogas generation. VW showed a worthier role than HD and S in the contribution of generating biogas. Obviously, from equation (7), the mixtures containing VW in major proportion and HD, S in minor proportion were having synergic effect with biogas yield being the highest. Rao, et al. [22] also found out the relationship between biogas yield and substrate composition in the mixture by a quartic regression model. However, the composition of the substrate in the mixture is merely external expression of chemical components inside.

Table 2. The significance of the regression coefficients

Coefficients	Estimate	T value	Pr (> t)
Intercept	53.7	0.933	0.4037
VW	744.8	7.529	0.0017
HD	192.2	2.846	0.0466
R-squared=0.9433; Adjusted R-squared =0.915; p-value=0.00321			

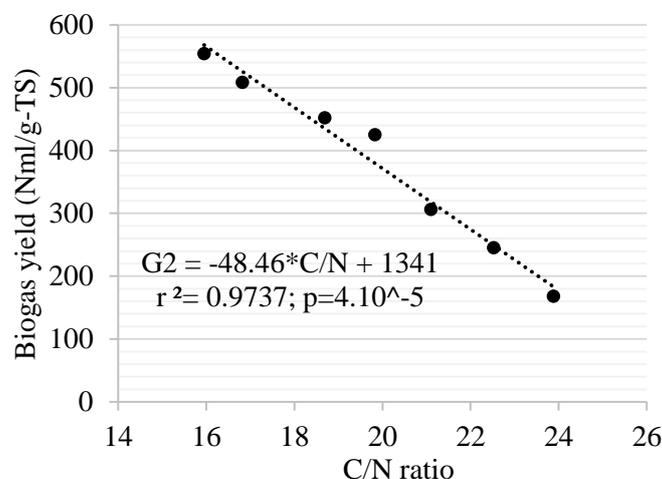


Figure 2. The relationship between biogas yield and C/N ratio.

3.2. Influence of C/N ratio to biogas yield

In essence, modification the proportion of each substrate in co-digestion aims to change nutrient ratio in the feedstock. In which, the nutrient quality is often evaluated indirectly by C/N ratio [17, 20]. The C/N ratio in the feedstock is too high pointing that the feedstock is not sufficient of nitrogen which needs for the build-up of microbial mass [3, 7]. Low C/N ratio leads to high concentration of generated ammonia, which is harmful to the AD processes [7]. Hence, the relationship between C/N ratio and biogas production was also investigated. Influence of C/N ratio on cumulative gas production was presented in Figure 2.

The high coefficient of determination demonstrated that C/N ratio is one of the key factors affecting the performance of the AD. The C/N ratio in the range of 16-23.9 showed a negative linear effect on biogas yield. The lower the C/N ratio, the higher the produced biogas. And an optimal C/N ratio was 16. This result agreed with the conclusion of some recent studies that low C/N ratio (15-20) was the best nutrient for the AD [8, 17].

3.3. Simulation of biogas accumulation

Both Gompertz (G) and (L) models were originally intended to describe the bacteria growth curve [12, 13]. With the assumptions of the rate of gas production is proportional both to the current microbial mass and to the substrate level, the L model - equation (6) is used to simulate biogas accumulation. The G model – equation (5) is also manipulated to express the biogas generation with the assumption that substrate limitations do not influence growth, and that the growth rate is proportional to the microorganism [13]. The kinetic constants A, μ_m , and λ of two models were determined by using the least squares fitting method (non-linear regression approach) with the aid of the solver function in MS Excel ToolPak. The received kinetic constants were shown in Table 3. By plotting simulation of the two models were also obtained the graph as shown in Figure 3.

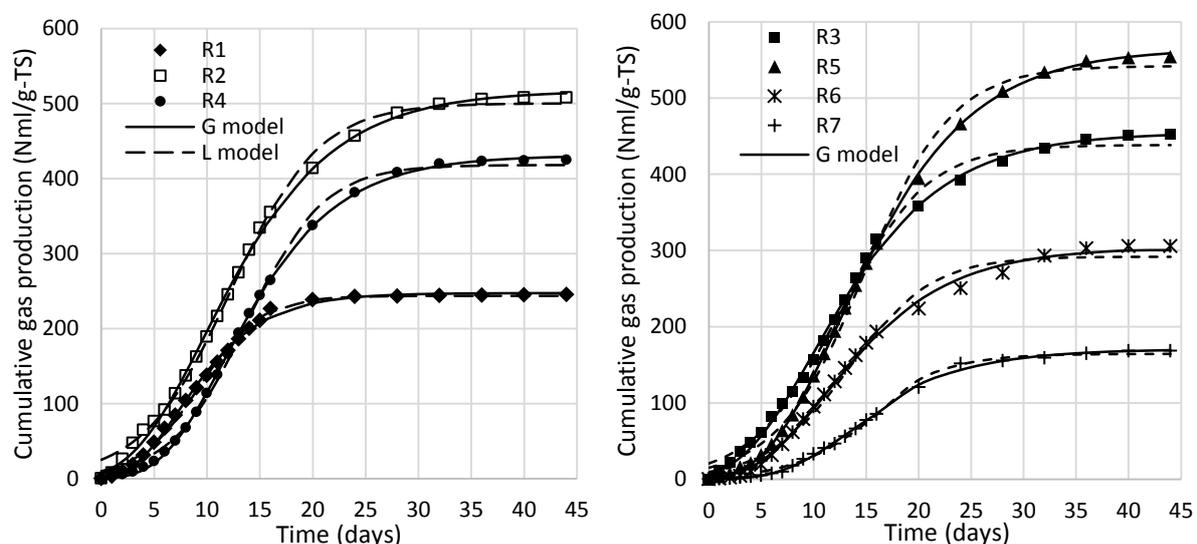


Figure 3. Accumulative gas production from experimental data and kinetic models.

The coefficient of determination was maximum in reactor R5 ($r^2=0.9999$) for G model and R4 ($r^2=0.9979$) for L model. However, the best SSE values were in reactor R7 for both models. The minimums of r^2 were 0.9977, 0.9901 in reactor R6 for G model and L model, respectively. Overall, results of the plotting data in Figure 3 and high coefficient of determination in Table 3 proved that both models were capable of simulating well the cumulative biogas production curve. However, the higher coefficient of determination and the lower sum of squared errors (SSE) demonstrated that the G model was better than the L model. This result had the same point of view with published reports from Latinwo and Agarry [11], Lo, et al. [23], and Lay, et al. [24].

Table 3. Constants of gas production kinetic models

No.	Modified Gompertz model					Modified Logistic model				
	A	μ_m	λ	R2	SSE	A	μ_m	λ	r^2	SSE
R1	247	21	2.91	0.9984	307	244	21	3.39	0.9972	637
R2	517	28	2.93	0.9987	997	500	30	3.92	0.9972	2322
R3	455	24	3.13	0.9982	1048	439	26	4.18	0.9963	2324
R4	431	27	5.67	0.9998	145	418	29	6.59	0.9979	3177
R5	565	30	5.44	0.9999	131	542	33	6.51	0.9966	3879
R6	304	17	4.54	0.9977	703	292	19	5.52	0.9901	3177
R7	171	10	6.91	0.9988	118	164	11	7.96	0.9969	339

There was different between the kinetic constants which were obtained from both models. The biogas production potentials (A) in G model were higher 1.5-4.2% than them in the L model. The G model showed the lower 1.7-10% values of μ_m , and also lower 16.6-33.8% values of λ . However, both models had the same expression, the biogas production (A and μ_m) reached maximum and minimum in the reactor R5 and R7, respectively for both models.

At time $t=0$ day, experimental data showed zero value of cumulative biogas while both models exhibited positive values for all reactors (see Table 4). However, the G model was still better than L model for simulation at zero points. For λ - lag time (days), which is often understanding as the min time taken to appear biogas. Experimental data exhibited that the biogas was generated as soon after $t=0$ (see Figure 3). While obtained λ -value changed in among 2.9-6.9 days in both models (see Table 3), many studies which used G and L models had displayed the same status [2, 11, 13, 25, 26]. Obviously, obtained λ -values did not reflect right itself definition.

Table 4. Cumulative biogas production at $t=0$ days

Model	R1	R2	R3	R4	R5	R6	R7
Experiments (Nml/g-TS)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G (Nml/g-TS)	1.3	7.7	6.1	0.4	1.6	1.1	0.1
L (Nml/g-TS)	9.9	24.7	20.3	8.9	15	8.7	2.9

4. Conclusions

Co-digestion of VW, HD, and S was performed successfully. Experimental results demonstrated that each single-substrate in mixture played a significant role in nutrient balance. And co-digestion of VW, HD, and S improved biogas yield from single-substrates. The high component of sludge in feedstock reduced biogas yield because of high C/N ratio. The high HD proportion in the mixture led to the high cellulose which was not good for the AD. Hence, the mixture contained VW in major proportion had synergic effect with the cumulative gas volume being the highest. The volume of the cumulative biogas (Nml/g-TS) could be estimated from the component ratio of substrates by equation $G1_{Nml/g-TS} = 53.7 + 744.8 \times VW(\%) + 192.2 \times HD(\%)$ ($r^2=0.9433$; p -value=0.00321), or from C/N ratio by equation $G2_{Nml/g-TS} = 1341 - 48.46 \times C/N$ ($r^2=0.9737$, p -value =0.000). While the equation G1 exhibited external phenomenon then the equation G2 showed internal essence of substrate proportion influences to AD.

The high value of correlation coefficient ($r^2=0.9901$ -0.9999) demonstrated that both the (G) and Logistic (L) models were appropriate to simulate the accumulation of biogas production. However, with higher r^2 -values and lower SSE-values in all reactors demonstrated that G model was better than L model. Influences of operating conditions such as temperature, pH, TS to the constants of the models were not investigated, that is a big limitation of this study.

4.1. Limitations of both models.

Experimental data showed both models failed at time $t=0$ day. The lag time - λ did not reflect the right itself definition, it seemed merely mathematical constant. In addition, a characteristic of biogas production curve $-t_0$ (when μ_m -biogas generation rate reached maximum) does not appear in the G and L models. Hence, it is difficult to make a comparison between experimental data and modeling data. That was the reason previous studies which focused on G and L models did not mention to t_0 -value. Overall, for these disadvantages in both models, there should be a complete model for an alternative.

Next study: We would like to try to find out a biogas production kinetic model that can overcome the limitations of conventional models which is mentioned above. As well-known, anaerobic digestion is defined as a series of processes including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Biogas is mainly including CO_2 and CH_4 , in which, methane is generated only from the methanogenic process, and CO_2 can be generated from acidogenic, acetogenic, and also methanogenic processes. Therefore, kinetic of biogas generation should include the two processes respectively.

5. References

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