

Application of monod two-substrate kinetics with an intermediate for anaerobic co-digestion of distillery wastewater and molasses/glycerol waste in batch experiments

K. Phayungphan^a, N. Rakmak^{a,b} and A. Promraksa^{a,b,*}

^a Department of Chemical Engineering, School of Engineering and Technology, Walailak University, Nakhon Si Thammarat 80160, Thailand

^b Biomass and Oil-Palm Excellence Center, Walailak University, Nakhon Si Thammarat 80160, Thailand

*Corresponding author. E-mail: archw.pr@mail.wu.ac.th

Abstract

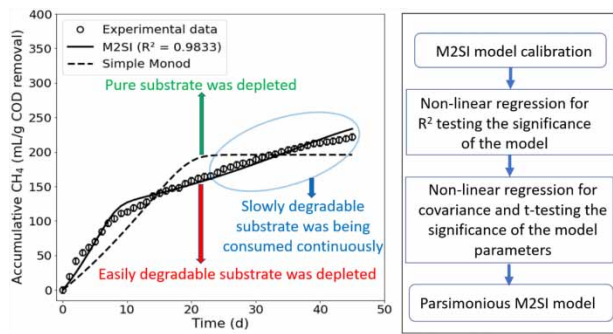
Anaerobic digestion is a highly complex process, particularly in co-digestion between poorly-defined, complex co-substrates like distillery wastewater, molasses, and crude glycerine. Thus, in this article, the authors tackled the problems by using Monod two-substrate with an intermediate (M2SI) model to represent accumulated biogas evolution (ABE) obtained from the co-substrates, including easily degradable, slowly degradable substrates and intermediate. The M2SI model predictions were compared with the traditional Monod model's simulation results to clarify an outstanding of the present model in the aspect of modeling and control. Different behaviors of ABE curves from batch experiments were used to calibrate the M2SI model prediction with sensitivity analysis of the model parameters. It was found that the M2SI model gives a correct trend to describe the co-digestion process with multiple substrates and complex microbial activities with satisfactory fitting accuracy. At the same time, simple Monod kinetics have a good fit for dilute pure distillery wastewater, but the estimated microbial growth kinetics were counterintuitive. Therefore, the M2SI Model has a broader range of applications for co-digestion dealing with the complexity of multiple microbial activities to consume inherently complex or artificial co-substrates.

Key words: anaerobic co-digestion, crude glycerol, distillery wastewater, intermediate, molasses, monod two-substrate kinetics

Highlights

- M2SI concept was proposed and calibrated with experimental data of co-digestion.
- M2SI was applied for the co-digestion of various ratios of distillery wastewater.
- M2SI model is more flexible in representing an ABE curve of complex co-substrates.
- Different microbial growth rates were investigated during anaerobic co-digestion.
- M2SI and Monod could give a similar interpretation for a pure and simple substrate.

Graphical Abstract



INTRODUCTION

In Thailand, local liquor and ethyl alcohol industrial processes are rapidly expanding. More than 2,600 distillery plants have been licensed for liquor or ethanol production. Liquors have been produced by fermentation from agricultural raw materials such as winery effluent, molasses, lemon juice, sticky rice, coconut sugar, and jaggery (Paschal *et al.* 2017; Ministry of Industry 2019). During the distillation processes, those distillery plants consume a large amount of water. Then approximately 30 m³/month (the concentrations of chemical oxygen demand (COD) in the range from 50 g/L to 104 g/L) of liquid effluents or distillery wastewaters (DW) have been discharged within their communities (Charnplakorn 2008). For the last few decades, researchers on alternative sources of renewable energy for environmental sustainability have been interested in and paid attention by scientists who studied sustainable/renewable energy production from biomass, particularly by anaerobic digestion (AD). The AD process can convert macro-molecular organic matter using microorganisms to methane and carbon dioxide (biogas), which helps to reduce dependency on fossil fuels (Fedailaine *et al.* 2015). The AD process is performed by microorganisms, especially bacteria; degradable organic matter in the absence of oxygen produces biogas, which typically consists of 60% to 70% CH₄, 30% to 40% CO₂, and small amounts of other gases (Weiland 2010). There are four main steps in the AD process: hydrolysis, acidogenesis, acetogenesis/dehydrogenation, and methanogenesis (Zeshan Karthikeyan & Visvanathan 2012).

Among the options for management of DW, AD is a practical, low-cost approach that offers many environmental benefits, such as the generation of renewable energy (methane and hydrogen), production of soil amendments, alcohol, volatile fatty acids, and other valuable materials, and contribution to decreasing emissions of the greenhouse gases (Wang Yang Feng Ren & Han 2012; Zeshan *et al.* 2012; Shen & Zhu 2016). However, DW can produce biogas only up to 10 L CH₄/L of DW because it typically has high nitrogen content and low C to N ratio (Charnplakorn 2008). The presence of high protein in DW causes ammonia accumulation and inhibits anaerobic microorganisms' activity in methane production (Menkveld & Broeders 2018). From adequate results by many research works, it is clarified that anaerobic co-digestion (ACoD) with other organic wastes such as agro-industrial wastes could achieve a nutrient-balance; that is, better C to N ratio and optimal pH as well as increase specific methane yield (Wang *et al.* 2012; Shen & Zhu 2016; Zahan Othman & Muster 2018).

Molasses (ML) is a significant by-product of the sugar industry. It is commercially available, cost-effective, contains a high concentration of sugars as well as other nutrients and can be used as a substrate in anaerobic digestion process for biogas production (Park Jo Park Lee & Park 2010). Crude glycerine or glycerol waste (GW) is the waste from biodiesel production. Generally, for every 100 kg of biodiesel production generated the GW is about 10 kg. The main composition of GW

contains 50% to 60% of glycerol, 15% to 18% of methyl esters, 12% to 16% of alkali, 8% to 12% of methanol, and 2% to 3% of water (Rivero Solera & Perez 2014). Glycerol waste is used in its pure form in many industries, such as pharmaceutical and cosmetics. GW's use is in smaller amounts compared with pure glycerine because its purification process is too expensive. Several researchers have used crude glycerine as a source of substrate for methane production, which is an alternative way to add value to the waste, which has high chemical oxygen demand and contamination from impurities (Amon *et al.* 2006; Yang Tsukahara & Sawayama 2008; Álvarez Otero & Lema 2010; Vásquez & Nakasaki 2016).

In the design of the biogas plant's optimal control systems, a full range of dynamic response of continuous anaerobic process is required. Carrying out full-range dynamic response experiments is prohibitively time-consuming and costly. From the literature, most studies have focused on co-digestion by mixing substrates at different ratios. Intensely few are working on the prediction of the dynamic response of continuous anaerobic co-digestion. This study's main objective is to predict methane production by using the most sensitive kinetic parameters from batch experiments for further design and operation of a continuous process. The relevant kinetic parameters of Monod two-substrate with an intermediate (M2SI) model will be obtained in this work to represent the ABE curves of methane production from different ratios of co-digestion between DW and other organic wastes. The simulation results of the two-substrate model were compared with simple Monod model prediction to describe complex microbial growth kinetics in the co-digestion processes. This article attempts to provide a better interpretation of the batch experiment data sets. This work aims to calibrate and obtain the most useful parameters of the two-substrate model from the co-digestion processes at a lab-scale to describe different microorganisms' activities to consume co-substrates in biogas production. Furthermore, when scaling-up the process to the production scale is required, it was anticipated that modeling in this work would accommodate a process design and operation for biogas production.

MATERIALS AND METHODS

Inoculum preparation

In this work, the inoculum at 35 °C of methanogenic bacteria was obtained from an anaerobic sludge at the bottom of the most active anaerobic pond from a palm-oil mill in Krabi province, Thailand. The inoculum was mixed with palm oil milled effluence (POME) at a percentage ratio of 20:80 by volume and the pH was adjusted in an appropriate range of 7 to 7.5 by adding 5 g/L of NaHCO₃ giving an equivalent alkalinity as CaCO₃ of 5 g/kg. The mixture was incubated at the controlled temperature for two weeks of biogas production; a gas counter measurement did not detect the amount of biogas, and the sludge should not be lower than 40 g/L for further use. The controlled inoculum was used for the biochemical methane potential (BMP) tests from the anaerobic co-digestion processes (O-thong Boe & Angelidaki 2012).

Experimental methods

The anaerobic co-digestion was performed in a 200 mL working volume batch reactor using the BMP assay. The digesters were operated using three substrates, including ML and GW (varying from 1% by volume to 5% by volume) mixed with DW at the designated ratio of substrates, as shown in Table 1. All substrates were adjusted by adding 5 g/L of NaHCO₃ to obtain a pH of 7 to 7.5. The inoculum (80% by volume, 15 g/L of VSS) was added to all digesters at a ratio of 20:80 of substrates to inoculum. Each batch experiment was repeated three times.

All digesters in Table 1 were operated in 500 mL glass bottles as reactors with a working volume of 200 mL. The bottles were covered with air-tight caps. Oxygen was first removed by flushing the bottle

Table 1 | Experimental design of anaerobic co-digestions

Digester number	Substrates 20% by volume		Inoculum 80% by volume
	DW (% by volume)	ML (% by volume)	
1	99	1	80
2	98	2	80
3	97	3	80
4	96	4	80
5	95	5	80
	DW (% by volume)	GW (% by volume)	
6	99	1	80
7	98	2	80
8	97	3	80
9	96	4	80
10	95	5	80

headspace with nitrogen. The temperature was maintained in an incubator at 35 °C. Digestion was continuously conducted for 45 d. The volume of biogas was measured daily by the water displacement method (Angelidaki *et al.* 2009).

Biogas yields and biochemical methane potentials

The composition of distillery wastewater, molasses, glycerol waste and inoculum used to start up the biogas reactors was characterized by their uncertainties as shown in Table 2 and calculation of the methane production at different co-substrates, and their ratios from experimental data from Luanunkarb's work (Luanunkarb 2016) are summarized in Table 3.

Table 2 | The characteristics of substrates used in the experiments

Parameters	Characteristics			
	Distillery wastewater	Molasses	Glycerol waste	Inoculum
pH	3.52 ± 0.02	4.95 ± 0.01	8.86 ± 0.01	7.61 ± 0.02
TS (g/L)	44.08 ± 0.60	914.50 ± 2.17	279.53 ± 3.34	81.26 ± 1.41
VS (g/L)	40.67 ± 0.58	671.97 ± 4.96	254.96 ± 2.94	48.48 ± 0.00
Ash (%)	0.34 ± 0.02	24.25 ± 2.79	2.46 ± 0.40	3.29 ± 1.56
COD (g/L)	57 ± 0.71	1,210 ± 2.83	2,925 ± 7.07	73 ± 0.14
Alkalinity (mg/L)	6.67 ± 0.02	583.33 ± 0.01	3,083.33 ± 0.01	33.33 ± 0.00
VFA (mg/L)	2,214.26 ± 0.01	9,470.62 ± 0.01	4,521.50 ± 0.00	280.39 ± 0.00
Carbohydrate (g/L)	28.68 ± 0.33	1,355.56 ± 5.23	22.47 ± 0.22	11.32 ± 1.11
Reducing sugar (g/L)	3.41 ± 0.96	267.92 ± 0.00	7.99 ± 1.94	9.98 ± 8.56
Lipids (g/L)	11.90 ± 0.97	46.78 ± 2.79	87.39 ± 2.82	1.33 ± 0.97
Hydrogen (%)	7.71 ± 0.09	5.83 ± 0.02	10.65 ± 0.01	N/A
Oxygen (%)	43.35 ± 0.04	37.55 ± 0.22	17.32 ± 0.27	N/A
Carbon (%)	47.06 ± 0.54	55.15 ± 0.04	72.03 ± 0.27	N/A
Nitrogen (%)	1.88 ± 0.02	1.46 ± 0.03	0.00	N/A
C/N ratio	25.03	37.77	100.00	N/A

Table 3 | Methane production data obtained from co-digestion of different ratios of two-substrate mixtures

Substrates	Accumulative methane (mL CH ₄)	Methane production (m ³ CH ₄ /m ³ wastewater)	Methane yield (mL CH ₄ /g COD)
100% DW (40 mL)	4,569.51 ± 5.0	114.24 ± 1.0	327.33 ± 16.3
99% DW +1% ML (by volume)	5,200.86 ± 5.0	130.02 ± 0.2	360.64 ± 10.2
98% DW +2% ML (by volume)	4,915.18 ± 5.0	122.88 ± 0.1	330.27 ± 7.0
97% DW +3% ML (by volume)	4,257.07 ± 5.0	106.43 ± 0.2	277.45 ± 12.2
96% DW +4% ML (by volume)	3,975.10 ± 5.0	99.38 ± 1.3	251.51 ± 11.1
95% DW +5% ML (by volume)	3,610.12 ± 5.0	90.25 ± 0.8	221.94 ± 10.3
99% DW +1% GW (by volume)	5,356.19 ± 5.0	133.90 ± 0.1	354.55 ± 4.0
98% DW +2% GW (by volume)	4,497.48 ± 5.0	112.44 ± 0.1	276.69 ± 2.4
97% DW +3% GW (by volume)	4,459.64 ± 5.0	111.49 ± 1.0	256.28 ± 15.1
96% DW +4% GW (by volume)	5,789.05 ± 5.0	144.73 ± 1.0	312.10 ± 8.3
95% DW +5% GW (by volume)	5,305.51 ± 5.0	132.64 ± 0.8	269.37 ± 10.0

Analytical methods

Biogas samples were analyzed by using gas chromatography. Sludge samples were collected from each digester to determine COD, volatile solids (VS), alkalinity, and volatile fatty acids (VFAs) were evaluated according to standard methods for the examination of water and wastewater (APHA 2017).

Kinetic model for prediction

A Monod two-substrate with an intermediate (M2SI) model was developed in previous work (Rakmak Noynoo Jijai & Siripatana 2019) for prediction of the dynamic response of biogas production based on the following assumptions:

- (i) Endogenous metabolism was included.
- (ii) A so-called intermediate S_i obtained from slowly degradable substrate S_s was added in the hydrolysis step and was consumed by a specific group of microorganisms (X_e).
- (iii) There were two groups of the organism: X_e (takes quickly degradable substrate S_e and intermediate S_i) and X_s (grows on S_s);

Based on the model assumptions, the mathematical two-substrate model dealing with a set of ODEs can be written as the following equations:

$$\frac{dX_e}{dt} = [\mu_e + \mu_i - k_{de}]X_e \text{ where } \mu_e = \frac{\mu_{me}S_e}{K_{Se} + S_e} \quad (1)$$

$$\frac{dX_s}{dt} = (\mu_s - k_{ds})X_s \text{ where } \mu_s = g(P) \left(\frac{\mu_{ms}S_s}{K_{Ss} + S_s} \right) \quad (2)$$

$$\frac{dS_e}{dt} = -\frac{\mu_e}{Y_{XeSe}}X_e \quad (3)$$

$$\frac{dS_i}{dt} = f_{isX} \left(\frac{1 - Y_{XsSs}}{Y_{XsSs}} \right) \mu_s X_s - \frac{\mu_i X_e}{Y_{XeSi}} \text{ where } \mu_i = \frac{\mu_{mi}S_i}{K_{Si} + S_i} \quad (4)$$

$$\frac{dS_s}{dt} = -\frac{\mu_s}{Y_{XsSs}}X_s + f_{SsX}(k_{ds}X_s + k_{de}X_e) \quad (5)$$

$$\frac{dP}{dt} = \frac{Y_{PSe}}{Y_{XeSe}}\mu_e X_e + \frac{Y_{PSi}}{Y_{XeSi}}\mu_i X_e \quad (6)$$

where X , S , P are the concentrations of microorganisms, substrates, and product (biogas), respectively. μ and μ_m are specific and maximum growth rates; k_d is the specific death rate; K_S is the half saturation constant; Y_{Xs} and Y_{Ps} are microorganism yield and product yield factors, respectively. The subscripts s , e and i represent slowly degradable substrates (S_s), easily degradable substrates (S_e), and intermediate (S_i), respectively; that is, K_{Ss} and K_{Se} are saturation constants of slowly degradable and easily degradable substrates respectively; k_{ds} and k_{de} the specific death rates of slowly degradable and easily degradable substrates respectively. Y_{XsSs} , Y_{XeSe} , Y_{XeSi} , Y_{PSe} and Y_{PSi} are the corresponding yield coefficients as specified by the subscripts. f_{SsX} and f_{isX} are conversion factors for $X \rightarrow Ss$ and $Ss \rightarrow Si$, respectively. Finally, the switching or preference function $g(P)$ as a function of P for the model prediction is defined as follows:

$$g(P) = \frac{1}{\pi} \left(\tan^{-1}(\kappa(P - P_c)) + \frac{\pi}{2} \right) \quad (7)$$

where

$$P_c = f_C(1 - f_{Ss})S_0Y_{PSe} \quad (8)$$

where κ is an amplifying factor and subscript c represents a critical value of individual parameters; for example, f_C and P_c , and f_{Ss} is the fraction of slowly degradable substrate in a mixed substrate. The simulations of simple Monod kinetics were performed to compare with the M2SI model. Equations (1), (3) and (6) were used for model fitting of simple Monod by using the two most sensitive parameters, such as μ_{me} and Y_{PSe} .

Model calibration methodology

The model calibration algorithm diagram to identify variable parameters is illustrated in Figure 1. Firstly, preliminary model fitting was performed to set a default or constant values as follows: $\mu_{mi} = 0.2\mu_{me}$, $k_{de} = 0.15\mu_{me}$, $k_{ds} = 0.1\mu_{me}$ (decay rate of biomass was reported in a range from 5% to 15% of the maximum growth rate, [Arzate et al. 2017](#); [Arzate 2019](#)), $f_{SsX} = 0.7$, $f_{isX} = 0.8$, $K_{Se} = K_{Si} = K_{Ss} = 10,000$ mg/L (the half saturation constants were reported in a range between 7,000 mg/L and 12,000 mg/L, [Bernard et al. 2001](#)). The biomass yield coefficients $Y_{XeSe} = Y_{XeSi} = Y_{XsSs} = 0.13$ mL/(mg/L) were fixed for all cases, $X_0 = 8,000$ mg COD was estimated by using the available value 2.67 g COD/g VSS of inoculum obtained from a pond of palm oil mill effluent ([Madaki & Seng 2013](#)). The initial substrate concentrations (S_0) and the fraction of slowly degradable substrates (f_{Ss}) can be manipulated based on the ratio of co-substrates. Yield coefficients of all substrates were assumed to be the same value for an individual case ($Y_{PSe} = Y_{PSi}$). Insignificant (tested by t-distribution of 99.9% confidence interval), manipulated or high coefficient correlation parameters in full M2SI model were identified to formulate reduced-parameter models according to the principle of parsimony. The experimental data were fitted and represented by Monod two-substrate with an intermediate (M2SI) model to compare with the simple Monod model. Non-linear regression to test covariance and t-distribution using the lmfit-module was available to import into the Spyder (3.7) environment with Python programming. Variable model parameters were identified, and M2SI model calibrations were performed by allowing lower and upper bounds for variable parameters. The models were implemented in the Python language coupled with the least square optimization routines to estimate the model parameters. The estimated parameters are given in Tables 4–6.

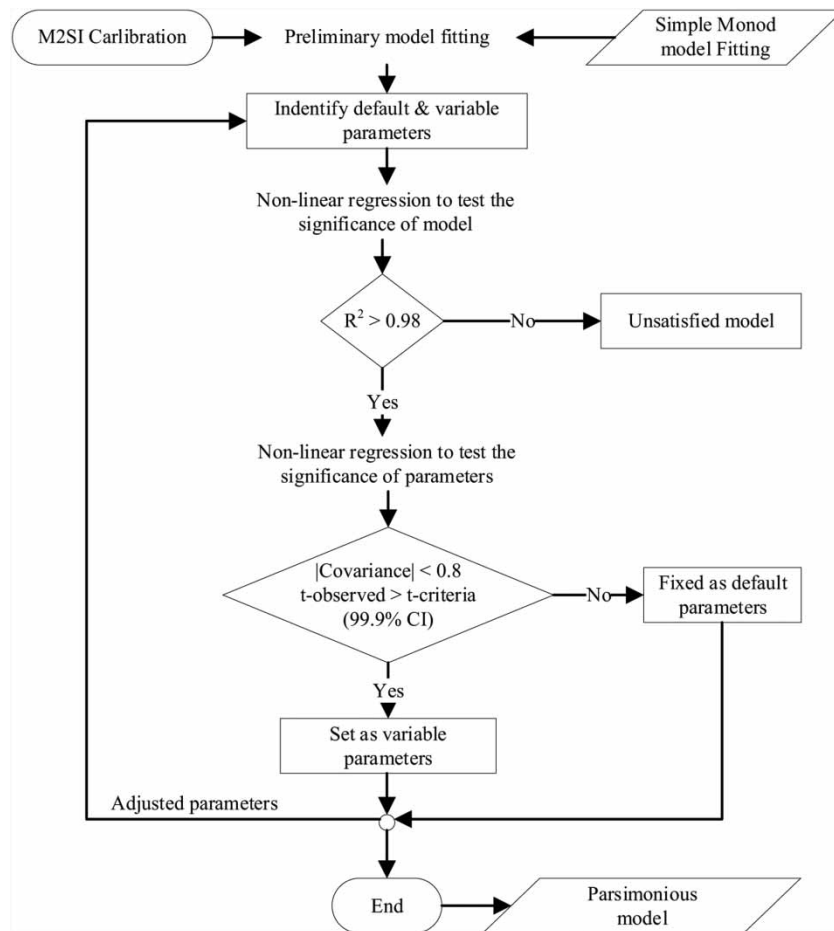


Figure 1 | Diagram for testing the significance of the M2SI model and the significance of the parameters.

Table 4 | Parameters used in the model prediction of accumulative methane production for different dilutions of single substrate distillery wastewater (DW)

M2SI parameters	Dilution ratios					
	50% DW	60% DW	70% DW	80% DW	90% DW	100% DW
$\mu_{me} (d^{-1})^*$	0.136 ± 0.015	0.117 ± 0.007	0.116 ± 0.008	0.161 ± 0.009	0.141 ± 0.005	0.162 ± 0.024
$\mu_{ms} (d^{-1})^*$	$(1.027 \pm 0.166)\mu_{me}$	$(0.63 \pm 0.064)\mu_{me}$	$(0.615 \pm 0.03)\mu_{me}$	$(0.528 \pm 0.002)\mu_{me}$	$(0.425 \pm 0.025)\mu_{me}$	$(0.68 \pm 0.057)\mu_{me}$
$Y_{PSc} (mL/(mg/L))$	0.048 ± 0.01	0.05 ± 0.003	0.045 ± 0.003	0.042 ± 0.002	0.045 ± 0.001	0.027 ± 0.005
$S_0 (mg\ COD/L)$	28,500	34,200	39,900	45,600	51,300	57,000
f_{ss} (no unit)	0.7	0.7	0.7	0.7	0.7	0.7
κ	0	0.02	0.01	0.016	0.016	0
f_C (no unit)	N/A	1.0	1.0	1.0	1.0	N/A
R^2	0.9851	0.9904	0.9967	0.9953	0.9962	0.9925
Monod parameters						
$\mu_m (d^{-1})^*$	0.031 ± 0.001	0.037 ± 0.001	0.04 ± 0.001	0.045 ± 0.002	0.047 ± 0.002	0.054 ± 0.002
$Y_{PS} (mL/(mg/L))$	0.011 ± 0.000	0.001 ± 0.000	0.008 ± 0.000	0.007 ± 0.000	0.006 ± 0.000	0.006 ± 0.000

*The parameter was obtained at the experimental temperature of 35 °C.

RESULTS AND DISCUSSION

The batch experiments were preliminarily done in the lab-scale digester to provide adequate information to describe bioreactions of a digestion process and to approach optimal control of the

Table 5 | Parameters used in the model prediction of accumulative methane production for different ratios of co-substrates between distillery wastewater (DW) and molasses (ML)

M2SI parameters	Ratios of co-substrates				
	99% DW + 1% ML	98% DW + 2% ML	97% DW + 3% ML	96% DW + 4% ML	95% DW + 5% ML
μ_{me} (d ⁻¹)*	0.2178 ± 0.02436	0.1588 ± 0.0082	0.1668 ± 0.0102	0.1825 ± 0.0091	0.1887 ± 0.0095
μ_{ms} (d ⁻¹)*	(0.3592 ± 0.0377) μ_{me}	(0.5003 ± 0.0219) μ_{me}	(0.3938 ± 0.0214) μ_{me}	(0.2123 ± 0.01) μ_{me}	(0.2052 ± 0.0101) μ_{me}
Y_{pSe} (mL/(mg/L))	0.0302 ± 0.0013	0.0235 ± 0.0007	0.0178 ± 0.0006	0.0202 ± 0.0004	0.0151 ± 0.0004
S_0 (mg COD/L)	68,530	80,060	92,160	103,120	114,650
f_{ss} (no unit)	0.85	0.75	0.7	0.7	0.6
κ	0	0	0	0.01	0.1
f_C (no unit)	N/A	N/A	N/A	1.0	1.0
R^2	0.9974	0.9972	0.9942	0.9941	0.9833
Monod parameters					
μ_m (d ⁻¹)*	0.0333 ± 0.0011	0.0432 ± 0.0015	0.0468 ± 0.0014	0.0644 ± 0.0028	0.0785 ± 0.0032
Y_{PS} (mL/(mg/L))	0.0053 ± 0.0002	0.0039 ± 0.0001	0.0034 ± 0.0001	0.0022 ± 0.0001	0.0017 ± 0.00003

*The parameter was obtained at the experimental temperature of 35 °C.

Table 6 | Parameters used in the model prediction of accumulative methane production for different ratios of co-substrates between distillery wastewater (DW) and glycerol waste (GW)

M2SI parameters	Ratios of co-substrates				
	99% DW + 1% GW	98% DW + 2% GW	97% DW + 3% GW	96% DW + 4% GW	95% DW + 5% GW
μ_{me} (d ⁻¹)*	0.1807 ± 0.0095	0.2127 ± 0.0055	0.2180 ± 0.007	0.1859 ± 0.0039	0.1924 ± 0.0038
μ_{ms} (d ⁻¹)*	(0.4441 ± 0.0197) μ_{me}	(0.4172 ± 0.0133) μ_{me}	(0.6702 ± 0.038) μ_{me}	(0.9193 ± 0.0211) μ_{me}	(0.6120 ± 0.027) μ_{me}
Y_{pSe} (mL/(mg/L))	0.0228 ± 0.0007	0.0147 ± 0.0002	0.0099 ± 0.0001	0.0096 ± 0.0001	0.0073 ± 0.0001
S_0 (mg COD)/L	85,680	114,360	143,040	171,720	200,400
f_{ss} (no unit)	0.60	0.57	0.54	0.52	0.50
κ	0	0	0	0	0.1
f_C (no unit)	N/A	N/A	N/A	N/A	1.0
R^2	0.9951	0.9950	0.9909	0.9952	0.9937
Monod parameters					
μ_m (d ⁻¹)*	0.0462 ± 0.0017	0.1004 ± 0.0044	0.1204 ± 0.0036	0.1148 ± 0.0026	0.1072 ± 0.0022
Y_{PS} (mL/(mg/L))	0.0038 ± 0.0001	0.0020 ± 0.0000	0.0016 ± 0.0000	0.0017 ± 0.0000	0.0012 ± 0.0000

*The parameter was obtained at the experimental temperature of 35 °C.

biogas production process. The ABE curves from batch mode were fitted by the Monod two-substrate kinetics with an intermediate to obtain the most effective model parameters for prediction of dynamic response (daily methane production) in further study on continuous biomethane production. In this study, different mixtures of co-substrates were used in a batch mode such as distillery wastewater (DW) in different dilutions, combinations of distillery wastewater and molasses (ML) at various ratios, and combinations of distillery wastewater and glycerol waste (GW) at different ratios. ABE data for each proportion of those dilutions or mixtures were fitted with the Monod two-substrate kinetics with an intermediate. Firstly, ABE curves of the experimental results with their reproductivity in terms of average standard deviation (SD) (represented by error bars of three repetitions) at different dilutions of DW were fitted, as shown in Figure 2, and the estimated parameters are summarized in Table 4. Secondly, ABE curves of the experimental results from using different ratios of two-substrate mixtures between DW and ML with their error bars were fitted, as shown in Figure 3, and the estimated parameters are summarized in Table 5. Finally, ABE curves of the experimental results from using different ratios of two-substrate mixtures between DW and GW with their error bars were

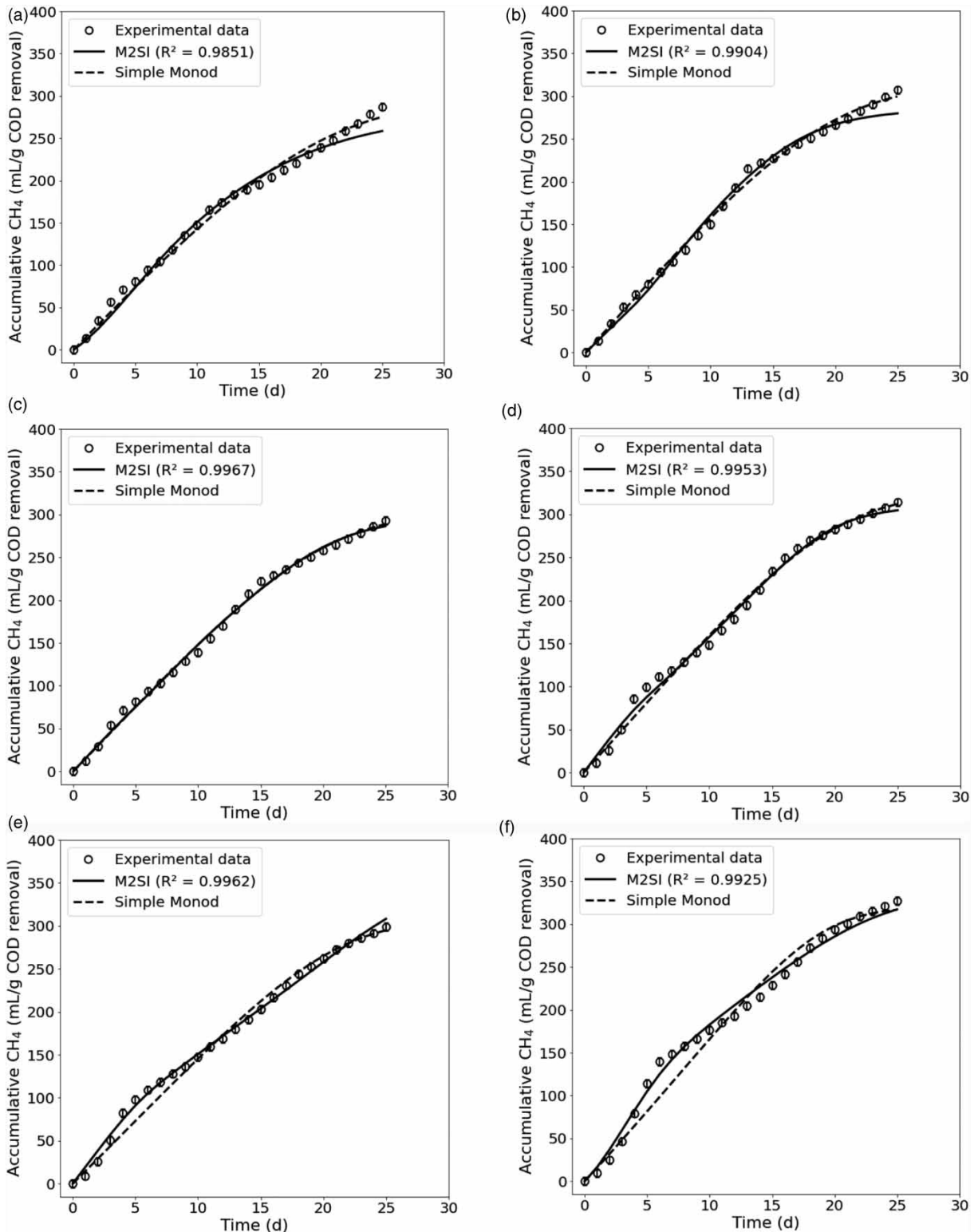


Figure 2 | Monod two-substrate kinetics model prediction for different dilutions of distillery waste (DW); (a) 50% DW, (b) 60% DW, (c) 70% DW, (d) 80% DW, (e) 90% DW and (f) 100% DW.

fitted, as shown in Figure 4, and the estimated parameters are summarized in Table 6. In Figure 2, a simple Monod model showed an adequate interpretation of microbial activities to consume a pure and easily degradable substrate. However, Monod model prediction ran off the experimental data tendency when the single substrate was getting more concentrated and too complex to be

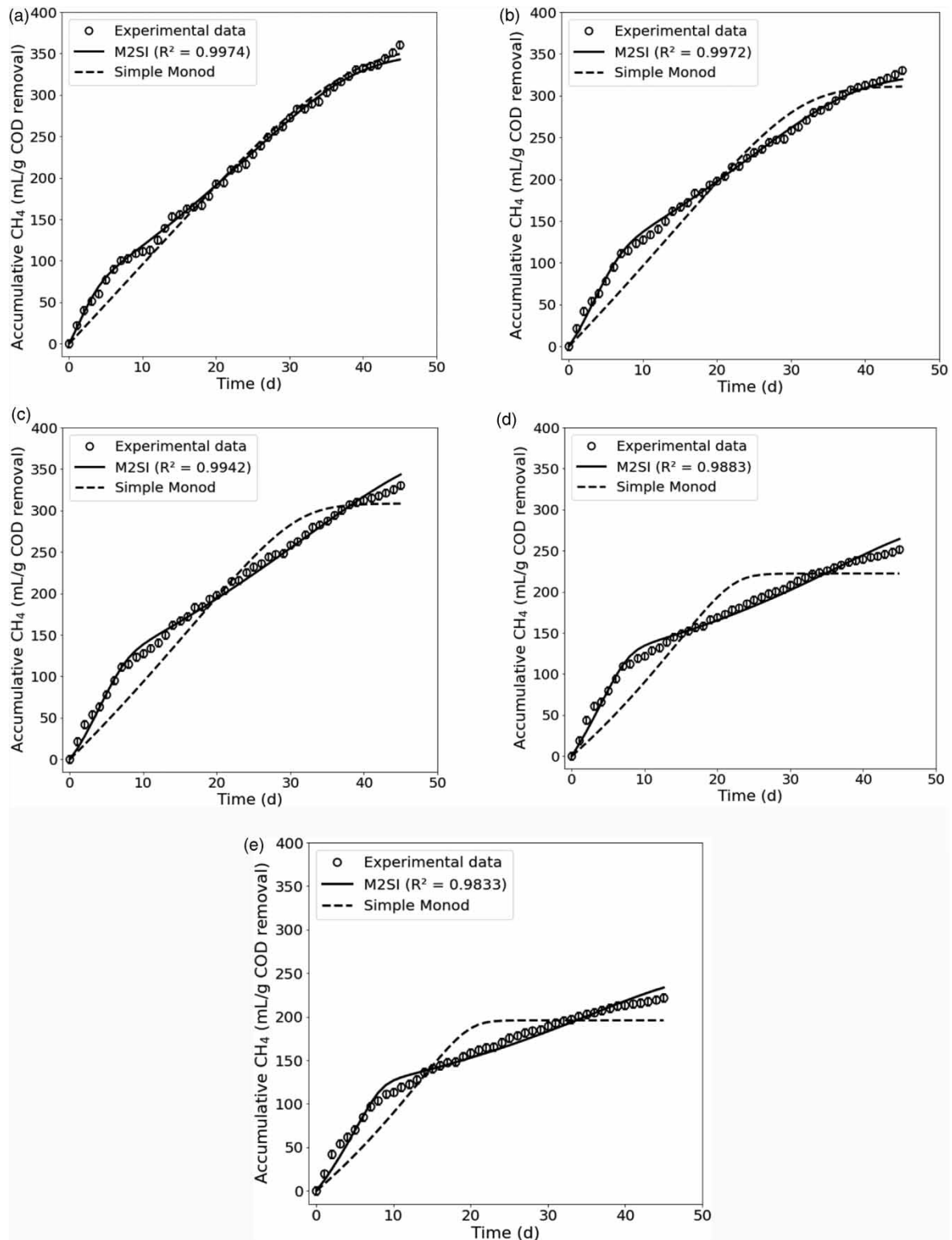


Figure 3 | Monod two-substrate kinetics model prediction for different ratios of co-digestates of distillery waste (DW) and molasses (ML) (a) 99% DW:1% ML, (b) 98% DW:2% ML, (c) 97% DW:3% ML, (d) 96% DW:4% ML and (e) 95% DW:5% ML.

consumed by a specific group of microbes. More distinct scenarios were found in Figures 3 and 4 when co-digestion between DW and other waste substrates in different ratios took place. It is seen in the co-digestion systems that M2SI is more flexible for interpretation for both series and parallel microbial activities to consume two different sources of nutrients. Conversely, simple Monod is not

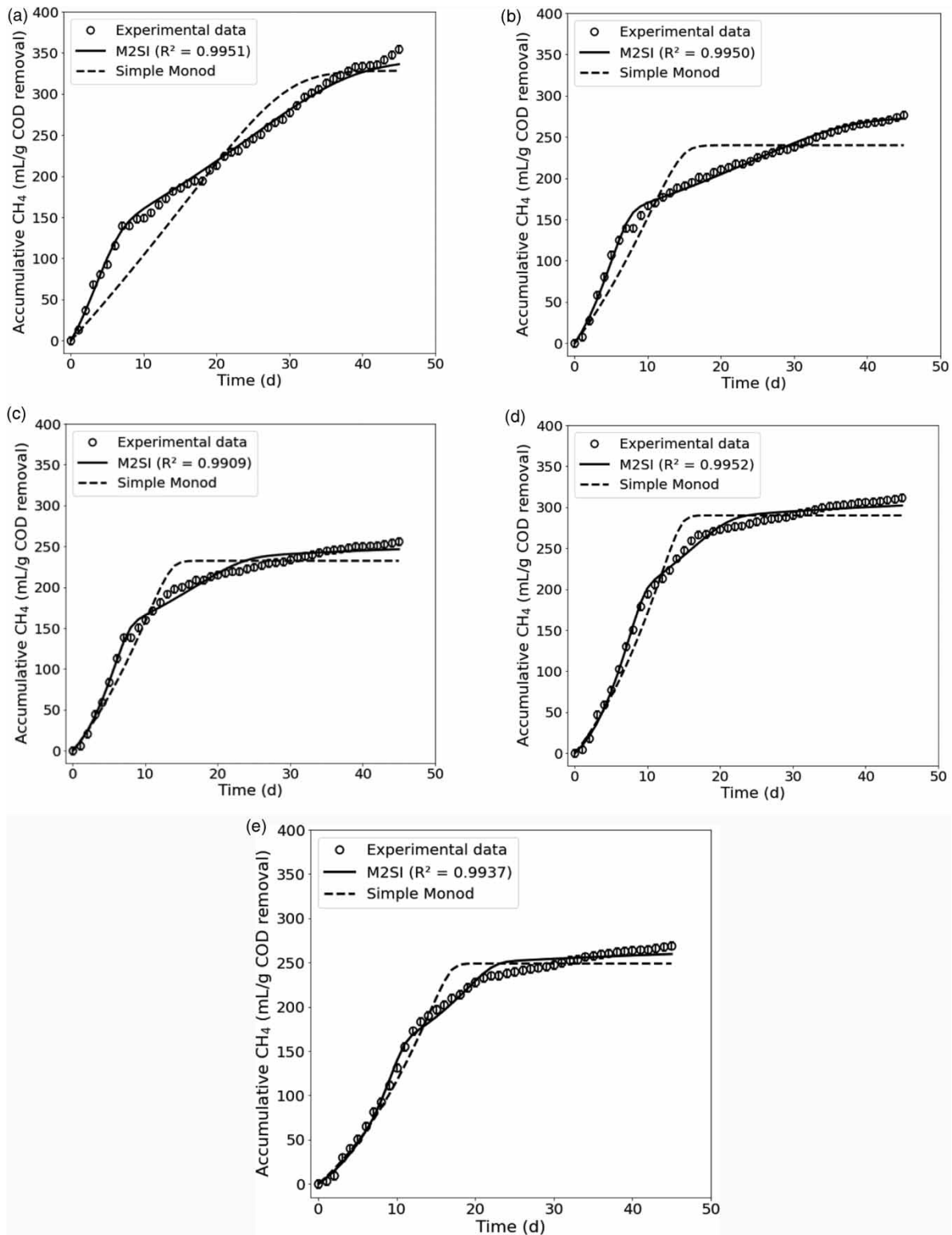


Figure 4 | Monod two-substrate kinetics model prediction for different ratios of co-digestates of distillery waste (DW) and glycerol waste (GW) (a) 99% DW:1% GW, (b) 98% DW:2% GW, (c) 97% DW:3% GW, (d) 96% DW:4% GW and (e) 95% DW:5% GW.

allowed to extend its application for digestion of complex co-substrates that may be consumed at different rates by individual groups of microorganisms. By the definition of the preference function, parallel microbial activities occur in a case without microbial preference ($\kappa = 0$ or $f_C = 1.0$) and this is the most cases found in this study. Microbial activities in series can be identified in the ideal

case of zero f_C where the easily degradable substrate was completely depleted, although it was not found in this study. The most complicated cases are series-parallel microbial activities, which are defined for sufficient substantial values of κ and f_C falling practically around the middle between 0 and 1.

From the estimated parameters in Table 4 for different dilutions of distillery wastewater (DW), it can be seen that DW was an inherently complex substrate since smaller values of μ_{me} (the maximum growth rate of microbes that prefer to consume easily degradable substrate) and larger values of μ_{ms}/μ_{me} were obtained compared with other co-digestions. It was implied that slowly degradable components were present in the wastewater to contribute an intermediate that was more pronounced because the maximum growth rate of a particular microbial group, μ_{ms} , for slowly degradable substrate consuming, was sufficiently large to affect the biogas production. The prediction trends from the M2SI model and the Monod model in Figure 2 have a similar trend in representing the ABE curves. However, the microbial growth rates obtained from the Monod model fitting are underestimated and misrepresent compared to the simulation results using Monod kinetics $\mu_{ms} = 0.153 \text{ d}^{-1}$ from a previous study (Sharma Ghosh & Ray 2016), which indicates that DW contained slowly degradable substrate. The result in Figure 2 shows that the prediction from the two models was consistent when DW was diluted further from 50% DW to 70% DW. However, the traditional Monod model fitting gave a worse representation of the ABE curves continuously when the substrate concentration was increased from 80% DW to 100% DW. It could be remarked that the Monod two-substrate kinetics with an intermediate has a broader range for the explanation of unusual and unexpected behaviors of a complex substrate being consumed by microbial activities with a satisfactory regression coefficient R-square (higher than 0.98).

From model fitting in Table 5 and their ABE curves of co-digestion between distillery wastewater and molasses, the use of Monod two-substrate kinetics with an intermediate showed better prediction than the use of simple Monod kinetics, with a regression coefficient R-square higher than 0.98. Furthermore, the estimated microbial growth rates from Monod model fitting are much smaller than the estimated microbial growth rates from the M2SI model and misconceive compared with a range from 0.1 d^{-1} to 0.5 d^{-1} obtained from a similar complex co-digestion in previous work (Jijai & Siripatana 2017). However, the M2SI predictions did not show pronounced preferences for consumed co-substrates by two different groups of microorganisms from their ABE curves since all types of degradation were dominated by the maximum growth rate of microbial activity to generate and consume an intermediate. The degradation rates were different but it could not be explicitly specified that biogas production was generated by which type of degradation due to the parallel microbial activities of the two different groups. This scenario is commonly found when $f_C = 1.0$ at the starting time of co-digestion (easily degradable substrate had not begun to be consumed). In the case of zero amplifying factors for high ratios of DW, the ABE curves showed a similar trend to the use of the single source DW. However, a single Monod type kinetics cannot give satisfactory interpretations for their evolution, especially at the end of processes where most microbial activities were taking on the slowly degradable substrate. Therefore we can imply from the prediction that distillery wastewater and molasses were consumed in parallel at different degradation rates by different groups of microorganisms involved in the biogas production.

Generally, co-digestion by different sources of nutrients; that is, co-digested between distillery wastewater and crude glycerine, in which different properties spontaneously affect biogas production performance, such as COD, the VFA to alkalinity ratio and the C to N ratio. For mixtures of the two different substrates, a two-substrate type of ABE curves were usually found from the experiments since one rate of biogas production no longer had a suitable explanation for these bio-processes. Sparse representations of ABE data were expected to be obtained if traditional Monod kinetics (single substrate) were used to describe the ABE curves. In contrast, the Monod two-substrate kinetics with an intermediate can be used to represent multi-substrate consumption rates of the curves with an excellent

regression coefficient (R-square) even higher than 0.98, as shown in Table 6. In this case, glycerol waste was the primary source of COD, but it was also obviously acting as easily degradable organic matter consumed by microorganisms, like molasses. The fraction of slowly degradable substrate (f_{ss}) is estimated from the model fitting trends to decrease with the increased amount of the waste substrate added. Consideration from the complexities of their ABE curves was generally included beside the maximum specific growth rate. According to this type of ABE curve, the two-substrate model with an intermediate, as proposed in previous work (Rakmak *et al.* 2019), played a crucial role in model prediction to get the most effective parameters in appropriate ranges to describe ABE data and to be more meaningful in further design of biogas operation and control in a continuous process.

CONCLUSIONS

Monod two-substrate kinetics with an intermediate were used to estimate the most significant parameters from the model's prediction to represent the ABE curves of methane production from anaerobic co-digestion in batch experiments. The experiments were classified by different dilutions or mixtures of co-substrates prepared between these substrates, including distillery wastewater, molasses, and glycerol waste. The results show that the two-substrate kinetics can be used in the prediction of ABE data in this study with calibration by experimental data of pure substrate dilution and co-substrates. It was calibrated and compared with the traditional Monod model simulation results to clarify an outstanding M2SI model in various aspects, such as a better representation of the ABE curves and a more sensible estimated parameter. The two-substrate model is very flexible to describe ABE curves from both single substrate and multiple substrate digestions, as shown by this work to represent accumulative methane evolution obtained from distillery wastewater alone or co-digestions with other organic waste substrates. In the DW digestion process, the M2SI predictions can be fitted very well for all dilutions from 50% DW to 100% DW without misrepresenting and the counterintuitive parameter estimated compared with the simple Monod fitting parameters. In the study of the co-digestion, the molasses acted like easily degradable organic matter in the substrate mixture with distillery wastewater, but they seem to be simultaneously degraded at different growth rates for different groups of microbes. Glycerol waste also acted like an easily degradable substance when co-digested with distillery wastewater, and required generation of an intermediate before being consumed for methane production. From the aspect of modeling and control, experimental data from batch mode are essential for further study of the evolution of methane production by a model prediction with reasonable representation. The two-substrate model has outstanding performance representing ABE data with a satisfactory coefficient of determination (R-square) and has a broader range of applications for co-digestion dealing with the complexity of spontaneous bioreaction. The most useful parameters obtained from the calibrated M2SI model could be further used to design an optimal control system with high accuracy.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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