



## **Non-linear kinetic modelling of anaerobic biodegradation of fruit and vegetable waste together with cooked oil**

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### **Abstract**

A series of laboratory experiments were conducted under anaerobic mesophilic conditions. Cumulative biogas production of co-digested vegetables (potato, carrot, and spinach), fruits (grape and orange) and mixture of fruits, vegetables and cooked oil collected from restaurants was determined. Experiments were conducted in laboratory reactors (6L) operated in batch mode with substrates organic loading rate ranging from 1.0 – 5.0 gVS/L. The kinetics constant “*k*” was determined using first order empirical exponential, Cone, Fitzhugh and Gompertz model with the aim of analysing the degradation performance and biogas production. Model’s fitting to the observed data were evaluated by calculating the Pearson product-moment correlation coefficient and the Root Mean Square Prediction Error. Furthermore, an anaerobic digestion dynamic model was developed in this paper. The results showed that all empirical models performed well comparatively with the observed data. Estimated “*k*” were similar for the vegetables and fruits co-digestion but significantly different in the case of the co-digestion with oil. The dynamic model gave a satisfying interpretation of the experimental process.

**Keywords:** Anaerobic co-digestion; kinetic models; Pearson correlation; Parameter estimation; Dynamic model.

### **1. Introduction**

The generation of solid waste in the Sultanate of Oman amounts to about 1.6 million tons per annum. Despite the continuous efforts conducted by the local government, currently, there is still solid waste being sent to landfills, thus adding to the atmosphere emissions of thousands of metric tons per annum of methane and carbon dioxide, the most adverse greenhouse gases. Moreover, landfilled waste generates considerable volume of leachate that percolates through the soil and pollutes the groundwater. Groundwater in Oman is seen as the first reliable source of water across the country. Therefore

deploying mechanisms to control groundwater depletion is one of the country first priorities.

Anaerobic Digestion (AD) is considered part of the possible solution to control excessive waste field dumping and therefore minimize the potential risk of groundwater contamination. It is considered as an optimal medium for transferring solid waste to green energy which offer a valuable alternative to excessively dumping of waste. Furthermore, AD is considered as a consolidated technology with more than 2200 high-rate reactors already implemented worldwide [1].

Fruit and vegetable wastes (FVW) are produced in large quantities in markets, and constitute a source of nuisance in municipal landfills because of their high biodegradability [2]. A possible way to dispose of these wastes is using the anaerobic digestion process. As a biological process, AD is characterized by a high degree of waste stabilization, low production of waste biological sludge, low nutrient requirements, no oxygen requirements, and production of methane which is a useful end product. AD process, as a powerful technique, has been applied to produce biogas from fruits, vegetables and food waste [3, 4, 5, 6]. In anaerobic digestion, co-digestion is the term used to describe the combined treatment of several wastes with complementary characteristics [7,8,9,10]. Nevertheless, an AD process is significantly dependent on the environmental conditions such as temperature, pH, nutrients content, carbon/nitrogen ratio (C/N) and carbon/phosphorus ratio(C/P), presence of inhibitors, substrate typology, micro-elements availability and particles size that can be responsible for undesirable drops in performance and even for detrimental failures [11]. Such environment conditions have a tremendous impact on the biogas production. In order to better understand the AD process one direction can be taken by studying the kinetics of methane production from feedstock(s). The determination of AD process kinetic parameters is important when designing and evaluating anaerobic digesters. First-order models are common models to describe the methane production from lignocellulosic materials as compared with soluble substrates [12].

In the first part of this paper four empirical models are proposed for studying the bioreactor kinetics of biogas yield from batch digestion of different substrates. The models will help in a clear and quantifiable manner, understanding the effects of mixing one or more substrates on the degradation kinetics. Statistical calculations such as *Pearson product-moment correlation coefficient (PCC)*, *residual analyses* and *Root Mean Square Prediction Error (rMSPE)* were conducted to determine the best model fit to the observed data (skills).

In the second part of this paper an estimation of the methane production, within an in-house developed dynamic model framework, is proposed. Dynamic model could be a useful tool to optimize co-digestion processes. Our proposed dynamic model is focused on mass-balances processes of the anaerobic digestion system and their kinetic description.

This work presents a novel and optimised procedure for analyzing biodegradability to better estimate kinetic parameters from batch experiments of individual substrates. Also, it contributes to the development of an accurate idea about the intermediate process going on inside the reactor.

## 2. Methods

### 2.1 Substrates

The fruit and vegetable wastes used in this study were collected from Al Mawalh Central Market in Muscat. The cooked oil was collected from nearby restaurants. All solid substrates were shredded in small pieces and stored at 4 degrees Celsius and characterized for Total solids (TS), Suspended solids (SS) and Volatile suspended solids (VSS)- (Table 1).

The cooked oil contains rich amounts of lipids. At higher concentration lipids are considered as problematic components for the good performance of an AD process [13]. Problems such clogging, adsorption to biomass (affecting the mass transfer process), microbial inhibition due to the degradation could be a trigger for enhancing the long chain fatty acids (LCFA) presence which increases the digester acidification process. Consequently, cooked oil is often co-digested with other substrate types to reduce the lipid concentration in the digester [9]. Various studies have shown that digesting materials with high-lipid content increases the methane yield [14]. In this paper, selected fruits and vegetables have been identified to be one of the co-digesting materials to be combined with oil waste. The encounter of high alkalinity substrates with cooked oil increases the reactor resistance to acidification due to fatty acid formation.

The substrates were characterised for Total Solids (TS), Suspended Solids (SS) and Volatile suspended solids (VS) as per APHA [15] methods.

### 2.2 Inoculum

Granular sludge obtained from a UASB reactor treating sugar factory effluent was used to inoculate the 6l volume bioreactor. The reactor was fed with 600-700 g of settled sludge and mixed well at  $35 \pm 5^\circ\text{C}$  to break down the granules. The inoculum was tested for its methanogenic activity by addition of 2 ml of ethanol a sole source of carbon, in a few batches. The characteristics of the substrates and inoculum are shown in Table 1.

Table 1 Substrates characteristics of and inoculum used in the experiments.

Parameters	Potato	Carrot	Spinach	Orange	Grape	Cooked-oil	Inoculum
Moisture content (%)	99.36	98.34	94.56	98.91	96.5	-	95.35
Total solids (g/g)	0.33	0.17	0.12	0.25	0.36	0.10	5.40
Volatile solids (g/g)	0.26	0.14	0.10	0.20	0.30	0.10	4.30

### 2.3 Reactor operation

The experiments were carried out in two identical double-walled reactors of 6-L effective volume: R1 and R2, maintained at 35°C by a regulated water bath. Mixing in the reactors was done by a system of magnetic stirring. The pH inside the reactor was continuously monitored online using Metler Toledo pH probe Inpro 4260i and maintained at 7.5±0.5. Reactors were operated in batch mode without withdrawal. Reactors R1 and R2 was fed with vegetable substrates at an OLR varying from 1.0 to 5.0 g VS/L, respectively.

### 2.4 Analysis methods

Total solids (TS) suspended solids (SS) and volatile suspended solids (VSS) were measured according to the standard method [15]. The biogas production was measured on-line every 2 minutes by Milligas counter MGC-1 flow meters (Ritter gas meters) fitted with a 4-20 mA output. The software RIGAMO, supplied by Ritter, was used to log the gas output. The samples

PCC was estimated by the following equation:

$$PCC = \frac{\sum_{i=1}^N (v_{i,mea} - \bar{v}_{i,mea})(v_{i,pred} - \bar{v}_{i,pred})}{\sqrt{\sum_{i=1}^N (v_{i,mea} - \bar{v}_{i,mea})^2} \sqrt{\sum_{i=1}^N (v_{i,pred} - \bar{v}_{i,pred})^2}} \quad (1)$$

where  $v_{i,j}$  is measured value of biogas production volume,  $\bar{v}_{i,j}$  is predicted value of biogas production volume, and  $N$  is number of measurements.

rMSPE was estimated as following:

$$rMSPE = \sqrt{\frac{\sum_{i=1}^N (v_{i,mea} - v_{i,pred})^2}{N}} \quad (2)$$

were centrifuged and the COD soluble was determined by spectrophotometry at 620 nm according to the HACH method (DRB-200, USA). The Volatile fatty acid (VFA) was determined by titration method.

### 2.5 Statistical analysis

After conducting the direct model validation (both visual and statistical) it is important to further analyze the accuracy of the model parameters, and to provide confidence intervals for the parameters and in turn, for the model prediction. The 95% ( ) confidence intervals for the non-linear least squares estimation of “k” were calculated. This means that: if the same population is sampled on numerous occasions and interval estimates are made on each occasion, the resulting intervals would bracket the true population parameter in approximately 95 % of the cases. A confidence stated at a (1- ) level can be thought of as the inverse of a significance level, The Pearson product-moment correlation coefficient was determined to measure the correlation magnitude between the measured values and the predicted values.

In order to compare evaluated models, rMSPE was calculated. The rMSPE values represent the deviation between predicted and measured values.

To compute asymptotic confidence intervals two (2) commands in Matlab software (R2012a) were used, which are, for parameters, *nlparci [beta, residuals, Jacobian]* for predicted value: *nlpredci [model, x, beta, residuals, Jacobian (J), simultaneous option, prediction option]*; *nlparci*'s Matlab function returns

$$CI_j = Z_{\frac{\alpha}{2}} \cdot \sqrt{\frac{\sum_{i=1}^N (v_{i,mea} - v_{i,pred})^2}{df}} \quad (3)$$

where  $Z_{\frac{\alpha}{2}}$  is the confidence coefficient,  $\alpha$  is the confidence level taken equal to 0.05 and  $df$  is the degree of freedom.

### 2.6 Kinetics models

Assuming first-order kinetics for the hydrolysis of particulate organic matter, the cumulative biogas

the 95% asymptotic confidence interval (CI) on the nonlinear least squares parameter estimates “beta”. Asymptotic confidence intervals for the two parameters were calculated using *nlparci*, and the confidence interval formula for each parameter ‘j’ used in our calculation is displayed here after:

production can be described by means of the following equations:

$$Bp = B_{max}(1 - \exp(-kt)) \quad \text{Exponential model [16] – (4)}$$

$$Bp = \frac{B_{max}}{1 + (kt)^{-n}} \quad \text{Cone model[17]– (5)}$$

$$Bp = B_{max}(1 - \exp(-kt)^n) \quad \text{Fitzhugh model[17] – (6)}$$

$$Bp = B_{max} \exp\left(-\exp\left[\left(\frac{e \cdot R_h}{B_{max}}\right)(\gamma - t) + 1\right]\right) \quad \text{Gompertz model [18-19] – (7)}$$

where  $Bp$  represents the predicted value of the biogas production as a function of time (t),  $B_{max}$  is the by-batch maximum biogas production (ml), “k” is the kinetic rate constant (1/h),  $R_h$  is the by-batch highest flow rate, e is equal to 2.71828 and  $\gamma$  is the length of the lag phase (h).

### 2.7 Estimation of model parameters

In order to have model to observation best fit, a mathematical methodology founded on the basis of sensitivity analysis and non-linear optimization is proposed. In order to identify models’ parameters a global mathematical criterion based on the optimization of the error between simulated values and measurements was applied. The model parameters

were estimated using the *nlinfit* and *optimist* functions in Matlab. A Levenberg-Marquardt algorithm [20, 21] for best least-squares estimation of non-linear parameters was applied in our calculations.

To avoid relying on one method approach for optimizing the determined parameters, several algorithms have been tested. They are search techniques that numerically approach the optimum parameter values by optimizing an objective function. After many tests, we found that the Levenberge-Marquard method (LMA) was the best one.

LMA usually starts using a steepest descent method and progressively becomes a Gausse-Newton method as it gets closer to the optimum value of the researched parameter. This way, the algorithm is more robust than Gausse-Newton but achieves better convergence than steepest descent. The literature review showed that LMA has been commonly applied to parameter identification in AD models. Garcia-Ochoa et al. [22] used LMA for the treatment of livestock manure. Aceves-Lara et al. [23] and Martin et al. [24] used LMA for raw industrial wine distillery vinasses. Deveci and Ciftci [25] used LMA for baker's yeast effluents analysis. Aceves-Lara et al. [23] combined with an asymptotic observer to evaluate the parameters kinetics.

### 2.8 Dynamic model

Because of the inherent complexity of AD processes, it is difficult to develop a mathematical model reflecting exactly the reality. Therefore, simplifications are necessary. One of the main utility of an AD model is its universality. Consequently, we propose in this paper an AD model treating biomass generally – it did not distinguish particular groups of microorganisms. This generalization could be justified by the fact that inoculum digested was applied, which contained mixed bacterial culture. In the model the substrate, intermediate (VFA) and final product (biogas) were defined by their concentration. The proposed model was based on the following stages: first stage, hydrolytic bacteria hydrolyzed the organic compounds into simple soluble compounds and then into volatile acids by acid forming bacteria. In the second stage, acetogenic bacteria lumped together with methanogenic bacteria converted volatile fatty acids into methane and VFA, which concentration was at a low level during the whole process.

AD processes co-digestions were described by the following system of differential equations:

$$\frac{dS}{dt} = -k \cdot S \quad (8)$$

$$\frac{dVFA}{dt} = \frac{Y_{VFA}}{S} \cdot k \cdot S - \mu_{VFA} \cdot \frac{VFA}{K_s + VFA} \cdot OLR \quad (9)$$

$$\frac{dCH_4}{dt} = \frac{Y_{CH_4}}{VFA} \cdot \mu_{VFA} \cdot \frac{VFA}{K_s + VFA} \cdot OLR \quad (10)$$

$$\frac{dCO_2}{dt} = \frac{Y_{CO_2}}{S} \cdot k \cdot S + \frac{Y_{CO_2}}{VFA} \cdot \mu_{VFA} \cdot \frac{VFA}{K_s + VFA} \cdot OLR \quad (11)$$

where  $k$  is the constant of first-order reaction ( $h^{-1}$ ),  $S$  is the substrate concentration (mg/L), VFA is volatile fatty acid concentration (mg/L),  $\frac{Y_{VFA}}{S}$  the yield factor of VFA from substrate,  $\mu_{VFA}$  is the maximum specific utilization of VFA rate ( $h^{-1}$ ),  $K_s$  is the saturation constant (mg/L), OLR is the biomass concentration expressed in organic loading rate units (mg VS/L),  $\frac{Y_{CH_4}}{VFA}$  is the yield factor of CH<sub>4</sub> from VFA,  $\frac{Y_{CO_2}}{S}$  the yield factor of CO<sub>2</sub> from S and  $\frac{Y_{CO_2}}{VFA}$  is the yield factor of CO<sub>2</sub> from VFA. Equations were solved using Euler forward method.

It is important to mention here that a quantitative approach to anaerobic digestion of co-digested waste demanded a more complicated model than the one we proposed in this paper. The VFA inhibition could be included via additional terms in the set of equations mentioned above.

## 3. Results and Discussion

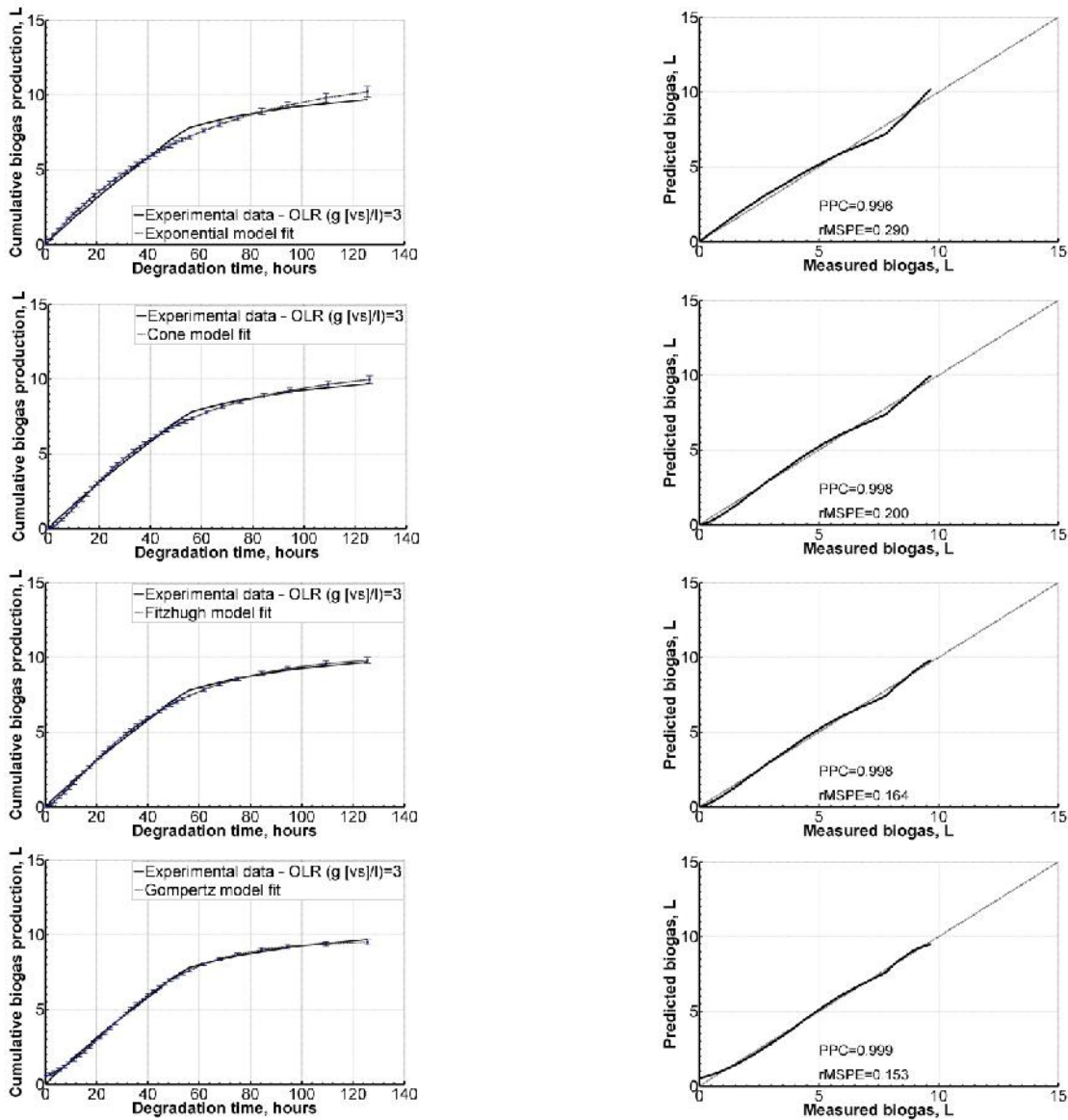
### 3.1 Empirical model's skills

There are different ways of verifying the models' capability of reproducing the observed results. One of the best and simplest is the visual inspection: if the model follows well the data evolution then it is behaving adequately in fairly reproducing the experiment. Historically, many papers used this technic to assess the model performance in AD systems, and it has even been the only applied method in many cases [26,27,28]. In the conducted batch assays (Fig. 1-3), from comparing the simulation and the

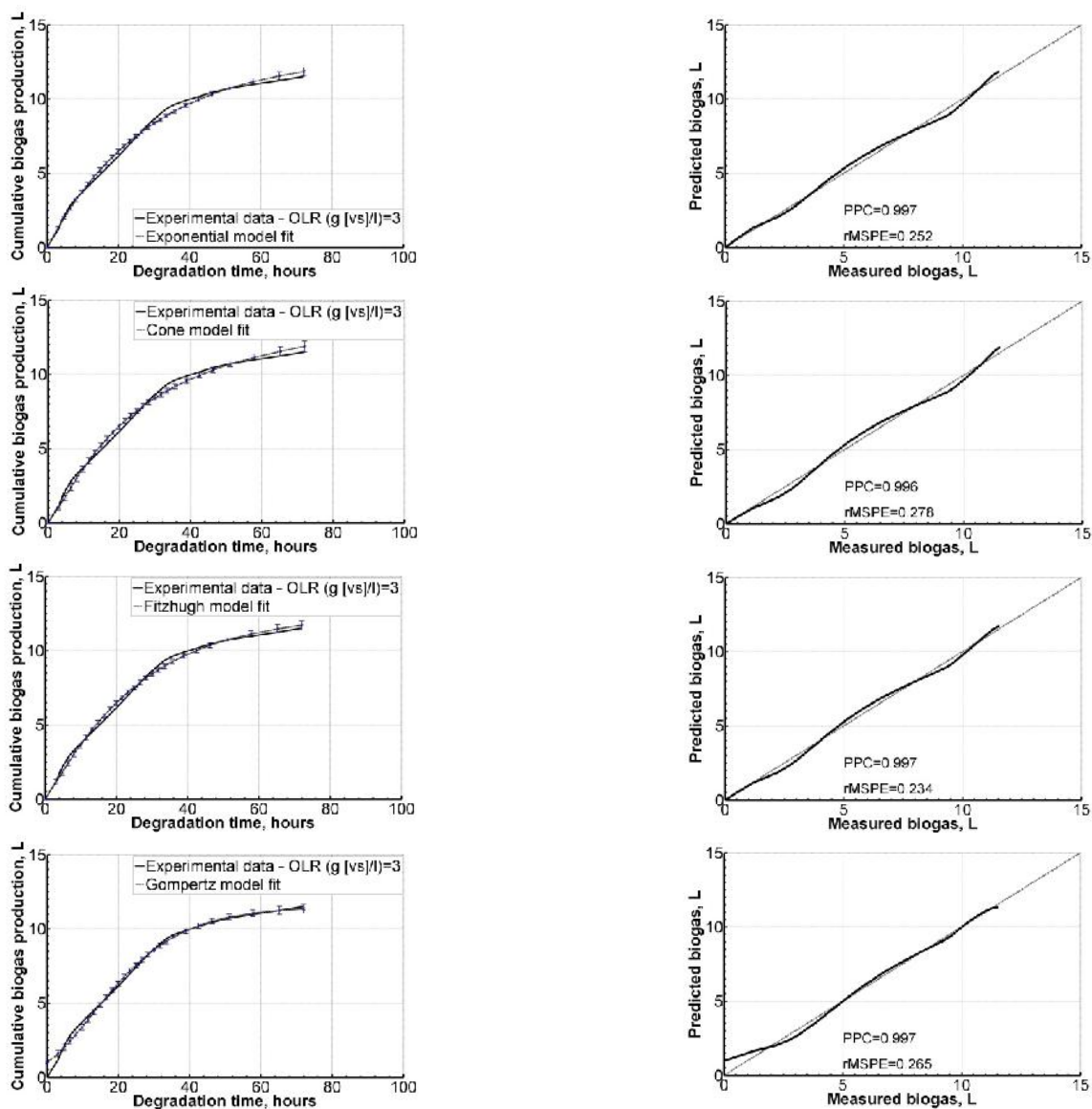


experimental results, it was obvious that the models' skills were encouraging. They fit very well to the experimental data. The correlation coefficients (*PCC*)

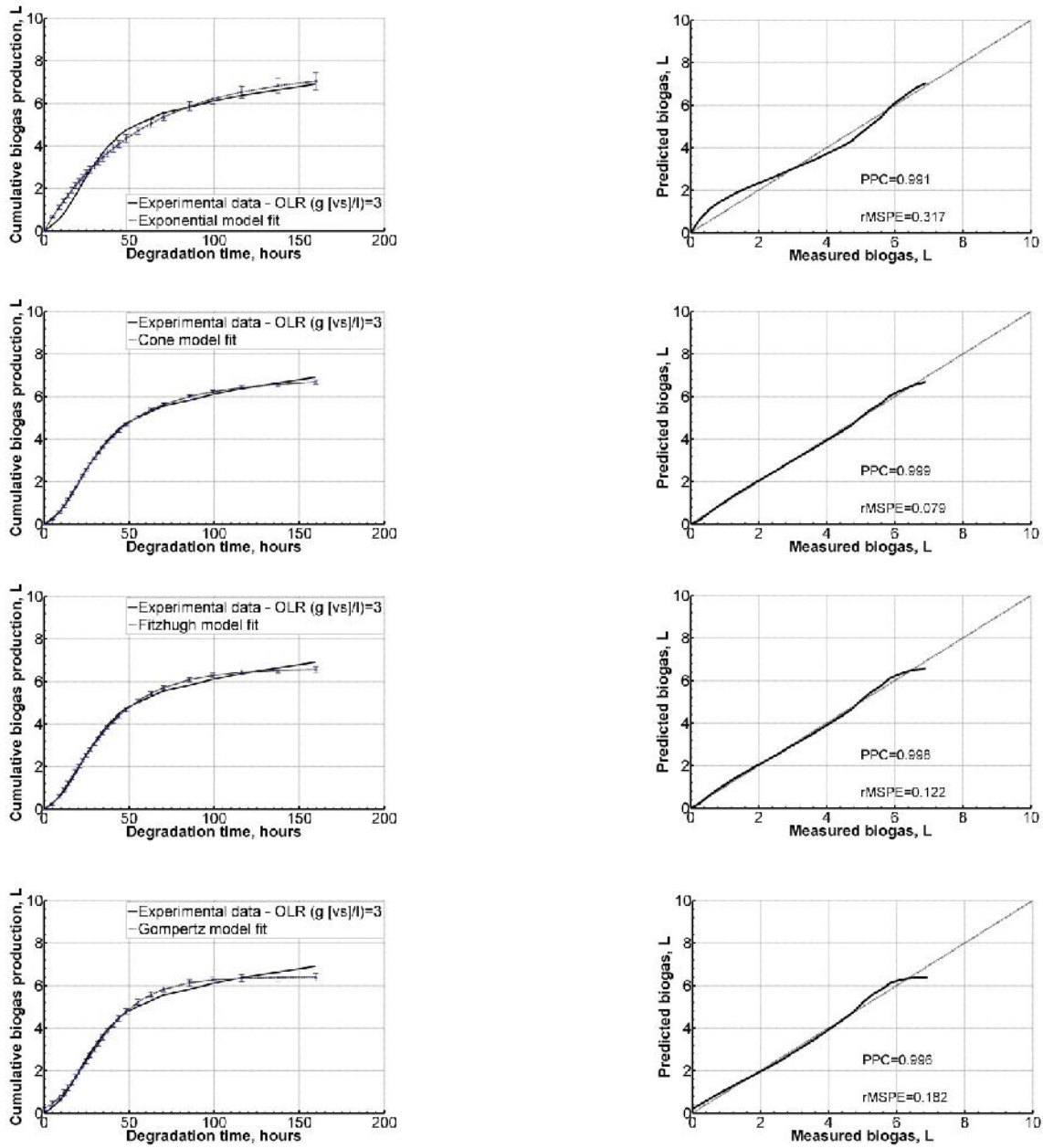
calculated for each test (Table 3) range between 0.996 and 1.0.



**Fig.1** (left plots) Simulated and experimental batch results for **co-digestion of vegetable waste**. (Dashed line) Empirical model. (Continuous line) Experimental results. (right plots) Predicted vs. measured cumulative biogas production for each batch assay.



**Fig. 2** (left plots) Simulated and experimental batch results for **co-digestion of fruit waste**. (Dashed line) Empirical model. (Continuous line) Experimental results. (right plots) Predicted vs. measured cumulative biogas production for each batch assay.



**Fig.3** (left plots) Simulated and experimental batch results for **co-digestion of fruit, vegetable and cooked-oil waste**. (Dashed line) Empirical model. (Continuous line) Experimental results. (right plots) Predicted vs. measured cumulative biogas production for each batch assay.

Results of rMSPE values calculated for each test - shown in Table 3 - clearly demonstrate that the test

with high value of *PCC* had the lowest value of rMSPE.



**Table 3** Pearson correlation coefficients (PCC) and Root mean square prediction error (rMSPE) of different models.

Models Parameters	Exponential		Cone		Fitzhugh		Gompertz	
	“PCC”	rMSPE	“PCC”	rMSPE	“PCC”	rMSPE	“PCC”	rMSPE
<b>Vegetables co-digestion</b>								
Test 1a	0.995	0.048	<b>0.997</b>	<b>0.036</b>	0.995	0.048	0.985	0.081
Test 1b	<b>0.997</b>	<b>0.111</b>	0.995	0.134	<b>0.997</b>	<b>0.111</b>	0.995	0.140
Test 1c	0.996	0.290	0.998	0.200	0.998	0.164	<b>0.999</b>	<b>0.153</b>
<b>Fruits co-digestion</b>								
Test 2a	0.999	0.036	0.999	0.036	<b>1.000</b>	<b>0.026</b>	0.995	0.092
Test 2b	0.998	0.120	0.999	0.072	<b>1.000</b>	<b>0.051</b>	0.989	0.294
Test 2c	0.997	0.252	0.996	0.278	<b>0.997</b>	<b>0.234</b>	0.997	0.265
<b>FVC co-digestion</b>								
Test 3	0.991	0.317	<b>0.999</b>	<b>0.079</b>	0.998	0.122	0.996	0.182

**NB.** Tests 1a-c are for co-digestion of vegetable waste feeding equal to 1-3 OLR respectively.  
 Tests 2a-c are for co-digestion of fruit feeding equal to 1-3 OLR respectively.  
 Test 3 is for co-digestion of fruit, vegetable and cooked-oil waste feeding equal to 3 OLR

Emphasis was given to the most critical value  $B_{max}$  which is the ultimate biogas production, i.e., the cumulated biogas produced at  $t = +\infty$ . All models yielded a reasonable estimate of  $B_{max}$ . If the predicted and experimental biogas production vary by 10% it is assumed that the experimental data does not fit the model and hence the value of “k” is invalid.

#### 4. Results

The estimated “k” parameters of the studied models are shown in Table 4. The calculated values of Pearson correlation coefficients and root mean square prediction error are shown in Table 3. Despite the highest values of PCC observed for all models, Table

3 demonstrates that the significant highest values of PCC (lowest values of rMSPE) could be calculated for both the Gompertz model results (highlighted values) with a few exceptions: the Cone model and Fitzhugh model had a slightly higher value of PCC respectively than the other models. The model with the lowest value of rMSPE is most likely to be correct.

Table 4 shows that the estimated values of “k” for all five models were almost constant for each of the tests while the estimated “k” values for both co-digestion of vegetable and co-digestion of fruits test were higher in the case of co-digestion of fruits, vegetable with cooked-oil.

**Table 4** Estimated “k” of the studied models (the 95% confidence intervals for the non-linear least squares parameter estimates “k” are between parentheses).

Test	“k”Exponential (1/h)	“k”Cone (1/h)	“k”Fitzhugh (1/h)	“R <sub>n</sub> ”Gompertz (L/h)
<b>Vegetables co-digestion</b>				
Test 1a	0.07(0.023)	0.09(0.046)	0.07(0.062)	0.07(0.046)
Test 1b	0.03(0.006)	0.04(0.015)	0.03(0.014)	0.09(0.021)
Test 1c	0.02(0.004)	0.03(0.005)	0.03(0.006)	0.16(0.012)
<b>Fruits co-digestion</b>				
Test 2a	0.12(0.013)	0.10(0.046)	0.10(0.026)	0.25(0.058)
Test 2b	0.08(0.019)	0.08(0.043)	0.06(0.021)	0.29(0.190)
Test 2c	0.03(0.005)	0.04(0.016)	0.04(0.014)	0.30(0.040)
<b>FVCO* co-digestion</b>				
Test 3	0.02(0.006)	0.03(0.002)	0.04(0.008)	0.12(0.020)

\*FVCO: fruit, vegetable and cooked-oil co-digestion

Almost similar values of final biogas production ( $B_{max}$ ) could be estimated for all co-digestion tests conducted from all evaluated models (Table 2). The slight difference in estimating ( $B_{max}$ ) was observed in some cases. The authors reasonably think that interpreting of

biogas production mathematical model parameters may be difficult in some cases, and models with several parameters may not allow comparison of different substrates composition.

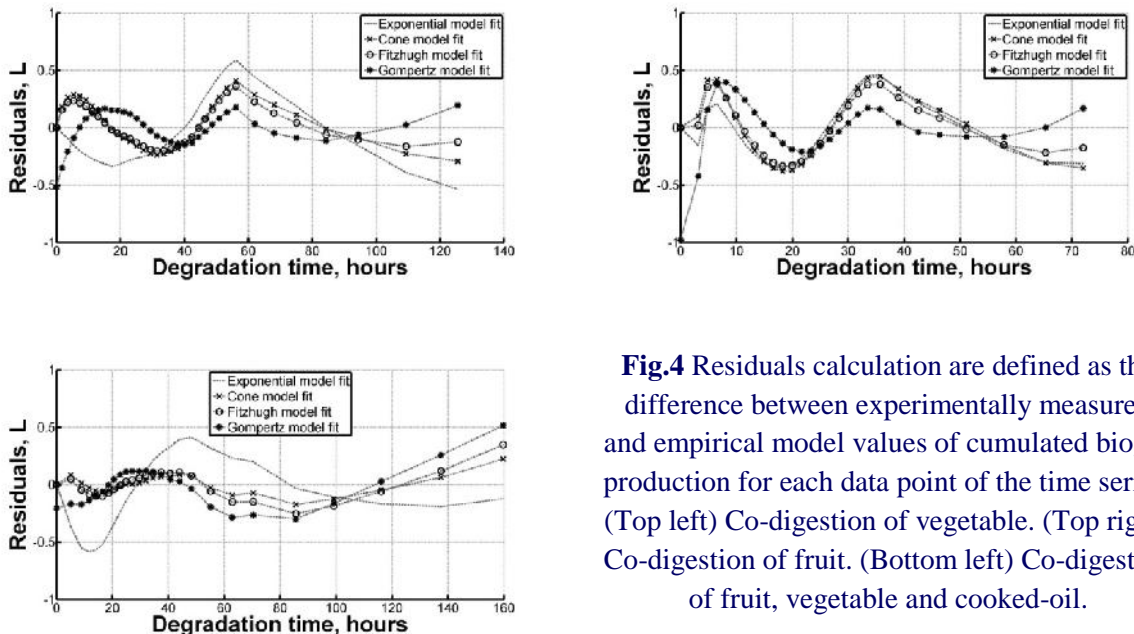
**Table 2** Total experimental cumulative biogas volume (L) vs. simulated biogas volume at different OLR

Substrate	Parameters	OLR (g VS/L)		
		1.0	2.0	3.0
Vegetable	Bp. Exp (L)	1.49(0.10)	4.47(0.07)	9.91(0.06)
	Bp. Simulated (L)	1.44(0.19)	4.45(0.35)	11.48(0.42)
	Duration (h)	57	200	126
Fruit	Bp. Exp (L)	3.21(0.07)	6.46(0.08)	11.68(0.06)
	Bp. Simulated (L)	3.41(0.19)	6.36(0.61)	12.88(1.03)
	Duration (h)	42	53	75
F.V. Oil	Bp. Exp (L)	-	-	7.08(0.08)
	Bp. Simulated (L)	-	-	7.45(1.19)
	Duration (h)	-	-	168

Figures 1-3 clearly show that all models did well in best fitting the experimental data but they did not well in predicting  $B_{max}$ .

Figure 4 displays residuals defined as the difference between experimentally measured and model values of cumulated biogas production for each data point of the time series. In a perfect model, residuals would be randomly distributed along time, meaning that the

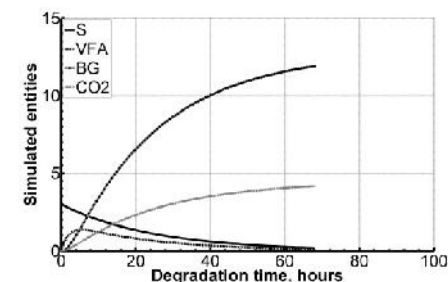
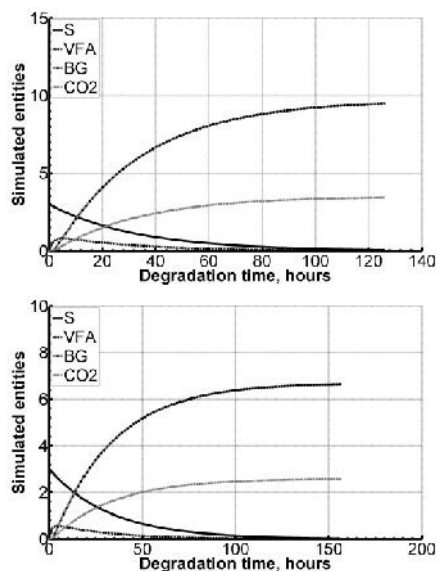
deviation of the empirical model from reality would be only related to measurement incertitude. This was not the case here, with the residuals peaking to the beginning and in the middle of the experimental period. The nonrandom distribution of the residuals implies that the models tested may not perfectly reflect the reality of the anaerobic digestion process: these models remain an estimate of the output of more complex biochemical processes.



**Fig.4** Residuals calculation are defined as the difference between experimentally measured and empirical model values of cumulated biogas production for each data point of the time series. (Top left) Co-digestion of vegetable. (Top right) Co-digestion of fruit. (Bottom left) Co-digestion of fruit, vegetable and cooked-oil.

One should notice that all four empirical models used in this work do not take account of the intermediates process prediction such as VFAs (Volatile Fatty Acids) and dissolved chemical oxygen demand. However, model such the mechanistic one has the ability to predict different intermediates and inhibitors

in anaerobic digesters. The proposed dynamic model enabled simulation of VFA concentration increase at the beginning of the process (as a result of hydrolysis) and further VFA decrease due to utilization in the biogas production (Fig. 5).



**Fig.5** Dynamic model simulated AD biogas (BG), substrates (S) and intermediate entities (VFA and CO<sub>2</sub>) for co-digestion batch assays. (Top left) Co-digestion of **vegetable**. (Top right) Co-digestion of **fruit**. (Bottom left) Co-digestion of **fruit, vegetable and cooked-oil**.

## 5. Conclusion

The models showed good performances. Strong positive PCC values were observed for all conducted tests (0.99). All four empirical models were distinguished by their highest skills for almost all conducted tests. The estimated “k” parameters were higher in co-digestion of fruits tests, co-digestion of vegetables tests than the case of co-digestion of fruits, vegetable with cooked-oil. The empirical models did well in best fitting the measurement plots but did not well in predicting  $B_{max}$ . This method may be used for evaluating the AD performance and better control of an AD system.

The simple mathematical model results showed good results comparatively to the experimental data. The model can be a useful instrument for the prediction of the process performance and the behavior of methane digestion.

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