

# Kinetic Modeling of Mixed Culture Process of Anaerobic Co-digestion of Vegetable Wastes with *Pistia stratiotes*: A Scientific Attempt on Biomethanation

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## Abstract

As co-digestion of two wastes together lead to balanced environment for biomethanation an attempt has been made in the present study to co-digest vegetable waste (VW) and *Pistia stratiotes* (PS) to overcome the acidification problem during mono-digestion of VW. Optimization of important process parameters for anaerobic co-digestion was done based on Central Composite Design (CCD) based Response Surface Methodology (RSM). Thereafter, modeling of the anaerobic digestion was carried out using first order, modified Gompertz and unstructured segregated model to study the process behavior. First order and modified Gompertz models were found to fit with the observed methane profile with  $R^2$  of  $\sim 0.99$ . In case of unstructured segregated model important kinetic constants such as  $K_{hs}$ ,  $K_{SS}$ ,  $K_{VFA}$  were derived from the model and the values were found to be  $0.1 \text{ d}^{-1}$ ,  $2.3 \text{ g/L}$  and  $881.68 \text{ mg/L}$ , respectively. Based on these kinetic parameters, simulated cumulative methane production was generated which matched well with the observed methane yield within the error band of  $\pm 20\%$ . This showed that the developed model could represent the overall process behaviour of this novel anaerobic co-digestion using mixed anaerobic culture as an inoculum.

**Keywords:** Vegetable wastes; *Pistia stratiotes*; Anaerobic co-digestion; Methane; Kinetic model

## Introduction

In recent times, anaerobic co-digestion has emerged as more prominent area of research since digestion of more than two wastes together provide more stability as compared to mono-substrate digestion [1]. The quantity of one type of organic waste generated at a particular site and duration may not be sufficient to make anaerobic digestion cost effective throughout the year. Fruit and vegetable waste often contain low quantity of nitrogen (1.2-1.4% (weight (w)/w) and high proportion of carbohydrates ( $\sim 58\%$  (w/w)) resulting in increased C:N that led to process instabilities like pH fluctuation and acidification of the reactor with subsequent reduction in biogas yield when used as a single substrate [2]. This necessitates balancing the C:N of vegetable waste (VW) by addition of nitrogen containing substrates. Co-digestion of two or more wastes supply missing nutrients in the substrate thereby it stabilizes the digestion process. Mata-Alvarez et al. have critically reviewed achievements of anaerobic co-digestion for improved biogas production that has been accomplished between 2010 and 2013 [3]. Several combinations of substrate mixture starting from animal manure to agro-industrial processing wastes have been widely reported. However, sporadic reports are available on utilization of aquatic weeds as a co-digestion substrate with other organic wastes. In the present study, a novel attempt has been made to utilize an aquatic weed *Pistia stratiotes* (PS) as co-substrate for anaerobic digestion of VW. According to the authors this is the first study to report anaerobic co-digestion of VW with PS for enhanced biogas production. PS is a fast growing aquatic weed widely distributed in Asia, North America and Australia which cause menace to the aquatic eco-system. It is characterized with large proportion of cellulose and nitrogen (2.59%, w/w) that can be co-digested with VW in order to balance the C:N for undeterred biogas production. An enhanced biogas production has been recently reported when PS was co-digested with industrial potato wastes [4]. Our preliminary laboratory investigation revealed that co-digestion of VW and PS in 1:1 weight proportion resulted in

methane yield of  $301.58 \text{ L/kg VS (Volatile solid)}_{\text{fed}}$  which is  $\sim$ two fold higher as compared to mono-digestion of VW ( $118.3 \text{ L/kg VS}_{\text{fed}}$ ). Sole substrate digestion of PS resulted in methane yield of  $450 \text{ L/kg VS}_{\text{fed}}$  and pH of the reaction medium turned alkaline (pH  $\sim 8.4$ ) with subsequent reduction in methane production due to release of ammonia during digestion process. This limits use of PS as sole substrate for digestion as it lead to ammonia accumulation in the anaerobic reactor followed by methanogenesis inhibition. In addition, PS is an invasive aquatic weed with scattered distribution in water bodies which may not be adequate to run a full scale functional biogas plant. On the other hand, as PS characterized with high nitrogen content it would be appropriate to use it as co-substrate with VW which is deficient in nitrogen and prone to acidification.

Upon ascertaining the co-digestion efficiency, an attempt has been made in the present study to optimize important process parameters such as substrate loading, co-substrate proportion and inoculum concentration for the anaerobic co-digestion of VW with PS through Central Composite Design (CCD) based Response Surface Methodology (RSM). RSM is based on the analysis of response as affected by several factors and its objective is to determine the optimal condition of the response. RSM provide the advantage of representing

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the predicted model in terms of regression equation which denotes the effect of individual factors and their interaction on the response that can be examined with lesser number of experiments. Zhu et al. determined the optimum level of different variables to optimize the microwave assisted extraction of astaxanthin from *Phaffia rhodozyma* [5].

Anaerobic co-digestion of organic wastes from different origin need precise management since random or heuristic decisions on the proportion of waste or feedstock to large scale plants might lead to process instabilities and significant reduction in methane yield [6]. Consequently, there is a requirement for accurate modeling of the anaerobic degradation of waste [7]. The advantage of employing mathematical models lies in their ability to reproduce dynamic process behaviour on a computer in a precise and quantifiable manner. The mathematical equations are used for simulating the physical, chemical and biological processes [8,9]. Modeling of the anaerobic degradation of real and complex wastewater has been studied by many researchers worldwide [7,10-12]. However, no unified modeling framework for the anaerobic digestion process exists so far except the Anaerobic Digestion Model (ADM) 1 model developed by International anaerobic modeling task group [13]. The mathematical models based on biological system improve the basic understanding of the system and their underlying mechanism which helps in formulation and validation of hypothesis, prediction of behaviour of the system under different situation and environmental conditions. This in turn reduces the risk factors, manual errors, time and requirement of experimental information.

There are two types of kinetic models namely structured and unstructured models which are being employed to study the behavior of the anaerobic digestion process. There were several reports available in the literatures that describe structured-segregated, unstructured-non-segregated, structured-non-segregated models for prediction of performance of the anaerobic digestion [13]. During anaerobic degradation of organic wastes, products such as soluble substrates, hydrogen, carbon di-oxide, organic acids, Volatile Fatty Acids (VFA) and alcohols were concomitantly produced. Therefore, it is considered as a complex multi-product process. Based on the environmental conditions, distribution of the intermediate degradatory products varies significantly. Under such unstable environment that prevails in a batch fermentation condition, it is not possible to establish structured models to explain such a multiproduct process whereas unstructured model might be a better option. Most of unstructured models were based on Monod or Contois kinetic equations. However, few studies have been carried out on unstructured models to explain the kinetics of biological methane production by mixed anaerobic cultures. The aim of this work is to develop a Monod based kinetic model for anaerobic co-digestion of VW+PS with Mixed Anaerobic Consortia (MAC) as an inoculum and derive kinetic constants which revealed the dynamic behavior of the process in each stages of anaerobic digestion. In addition, standard kinetic models such as first order and modified Gompertz were evaluated for their suitability of application to the selected substrates (VW+PS) and MAC. Experimental data from optimization studies were used for evaluation of kinetic models.

## Materials and Methods

### Substrates and inoculum

**Vegetable wastes:** Selection of wastes from the kitchen refuse was done based on the quantum of waste generated from processing and the usage routine of the vegetable. In the present study onion peels, cabbage, cucumber and cauliflower wastes were selected as the substrates for anaerobic digestion. These wastes were collected in bins and emptied

every 3 days so that an ample amount of wastes were available for anaerobic digestion process at regular intervals. After segregation, these four wastes were mixed in an equal proportion of 1:1:1:1 followed by maceration in a mechanical mixer grinder to make it slurry form. After characterization it was stored in 4°C until further use.

***Pistia stratiotes*:** The aquatic weed, *Pistia stratiotes* was collected from the local ponds of Kharagpur, West Bengal, India. This free floating weed is characterized with extensive hairy root system in which soil, inhabiting insects and debris were found to adhere to it. After hand removal of weeds from the water bodies, the plants were thoroughly washed under running tap water. For utilizing it as an additive for anaerobic digestion of VW, the plants were air dried and milled to form a powder with particle size of 0.5 mm (Table 1).

The characteristics of the chosen substrates have been represented in Table 1.

**Inoculum:** With the aim to replace the conventional inoculum sources like animal manure and digested sludge where availability in large quantity is bottleneck for large scale implementation, an attempt has been made in the present study to utilize specifically isolated MAC as an inoculum for biomethanation. This inoculum is a consortium of anaerobic bacteria comprising *Corynebacterium nuruki*, *Aneurinibacillus migulans*, *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Methanosarcina barkeri* and *Methanosaeta* sp. obtained from Microbial Biotechnology and Downstream Processing Laboratory, Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur, India. This inoculum was well maintained in the laboratory with appropriate feeding of nutrients and adaptation to the treatment of organic wastes. Specific Methanogenic Activity (SMA) of the inoculum was calculated according to the standard protocol and found to have a potential of generating  $0.41 \frac{g\ COD_{CH_4}}{g\ VSS^*d}$  [14]. This inoculum has been reported to be efficient in biogas production from pineapple wastes and potato wastes where the yield is at par with that of conventional source like cow dung [6,15].

## Methods and Materials

### Analytical procedure

Proximate analyses of the substrates were done based on the standard American Public Health Association (APHA) protocol [16]. Total carbon and nitrogen were determined from the elemental analyzer (Elementar vario Micro-cube, GmBH, Germany). Methane content of the biogas was estimated through gas chromatograph (Agilent 6820, Agilent Technologies, USA) equipped with HP PLOT (Porous Layer

Parameters	Units	Vegetable waste	<i>Pistia stratiotes</i>
	Moisture	(% of WW <sup>a</sup> )	91.50 ± 1.50
Total solid	(% of WW <sup>a</sup> )	08.50 ± 3.30	10.00 ± 3.72
Total volatile solid	(% of DW <sup>b</sup> )	91.00 ± 1.30	86.00 ± 1.70
Ash	(% of DW <sup>b</sup> )	09.00 ± 2.30	14.00 ± 3.40
Total carbon	(% of DW <sup>b</sup> )	44.30 ± 2.50	35.80 ± 1.71
Total nitrogen	(% of DW <sup>b</sup> )	01.66 ± 0.03	2.59 ± 0.02
C:N	-	26.69	13.80
Phosphorus	(% of DW <sup>b</sup> )	0.40 ± 0.02	0.20 ± 0.02
Potassium	(% of DW <sup>b</sup> )	1.07 ± 0.06	0.93 ± 0.05

<sup>a</sup>WW: Wet Weight Basis; <sup>b</sup>DW: Dry Weight Basis

**Table 1:** Characteristics of the substrates.

Open Tubular)-Q column and Thermal Conductivity Detector (TCD) having nitrogen as carrier gas. The temperatures of inlet and column (oven) were 40°C whereas detector temperature was set at 200°C.

### Optimization of process parameters

**Experimental design and set up:** In the present study, three important process parameters viz., substrate (VW) loading (g Total Solid (TS)/L), co-substrate (PS) proportion (% TS, w/w) and inoculum concentration (% VS/VS) were chosen for optimization. CCD was employed for design of the experiments with the chosen parameters (independent variables) each at three levels (-1, 0 and +1). Suitable range of process parameters for experimental design was selected from the preliminary experiments based on 'one variable at a time' approach (Table 2). The range of the parameters chosen for the experimental design has been represented in Table 2.

A total of 20 experiments (8 factorial points, 6 axial points, 1 centre point and its 5 replicates) were conducted based on the run order generated from Design Expert Software (version 9, Stat-Ease, Inc., Minneapolis, MN, USA). The purpose of conducting replicate of centre point is to obtain good estimate of experimental error. Cumulative methane yield (L/kg VS<sub>fed</sub>) was fixed as the response (dependent variable). All the experiments were carried out in triplicates under batch condition in serum vial of 125 mL capacity with 100 mL working volume. After addition of substrate and inoculum, the vials were sealed with butyl rubber cork and aluminium crimps under constant supply of N<sub>2</sub>:CO<sub>2</sub> (80:20) in an anaerobic chamber. The vials were incubated at 37°C under static condition. Mixing of reactor contents was done manually twice a day in order to avoid phase formation. Biogas produced was periodically measured for every 3 days by downward displacement of water (acidified to pH 2). Anaerobic digestion was carried out until no significant gas production was observed.

### Statistical Analysis

RSM was adopted for optimization of the chosen parameters to obtain maximum methane yield. The dependent variable i.e., Methane yield (L/kg VS<sub>fed</sub>) was fitted with a second order polynomial quadratic equation to correlate with the chosen parameters (independent variables) (Eq 1).

$$Y = N_0 + \sum_{i=1}^k N_i x_i + \sum_{i=1}^k N_{ii} x_i^2 + \sum_{i=0}^k \sum_{j=1}^k N_{ij} x_i x_j \quad (1)$$

Where, N<sub>0</sub> is the constant term; N<sub>i</sub>, N<sub>ii</sub> and N<sub>ij</sub> represent coefficients of linear (x<sub>i</sub>), square/quadratic (x<sub>i</sub><sup>2</sup>) and interactive (x<sub>i</sub>x<sub>j</sub>) terms. Y is the response i.e., dependent variable (cumulative methane yield).

The effect of individual factors and their interactive action on methane yield were analyzed statistically using significance analysis (t-test and F-test) and Analysis of Variance (ANOVA). The model terms were selected based on the p-value at 95% confidence level. The factors having p-value ≤ 0.05 were considered to be significant. 3D surface plots were generated to examine the effect of two factors on the methane yield. Moreover, efficiency of the model was assessed

Parameters	Units	Level of the factors (coded values)		
		-1	0	+1
Substrate loading (A)	(g TS/L)	5	7.5	10
Inoculum concentration (B)	(% VS/VS)	60	75	90
Cosubstrate proportion (C)	(% TS, w/w)	20	40	60

TS: Total Solid; VS: Volatile Solid; w/w: weight/weight

**Table 2:** Range of parameters (independent variables) for the CCD design.

from the coefficient of determination (R<sup>2</sup>). Finally, using response optimizer function (point optimization) optimum process parameters for maximum methane yield were obtained.

### Kinetic modeling

The profile of methane production obtained from the validation experiment (with the optimized process parameters) of CCD-RSM was used for kinetic modeling of anaerobic co-digestion process. The adopted kinetic models have been briefly explained in the subsequent sub-sections.

#### First order kinetic model

Preliminary analysis of methane production rate and maximum production potential from anaerobic digestion process was conventionally done based on first order kinetic modeling. Methane production follows the microbial growth pattern where there is a characteristic increasing and decreasing limb indicated by exponential and linear equation. In addition, the methane production from any organic wastes is proportional to the substrate degradation that has been found to follow the first order kinetic model [17,18]. The residual organic material in the biogas digester after biomethanation process can be predicted by considering the fact that the maximum methane production is proportional to the degradation of organic component present in the waste. First order kinetic model was frequently used in simulation of methane production because of its simplicity and requirement of less operational data. Thus in the present study to get an idea about the kinetic parameters such as maximum methane yield (L/kg VS<sub>fed</sub>) and rate constant, first order kinetic model was employed. The first order expression for methane production has been represented (Eq 2).

$$M = M_u (1 - e^{-kt}) \quad (2)$$

Where, t is the time of incubation, (days); M<sub>u</sub> is the ultimate/maximum methane yield obtained from given quantity of substrate, (L/kg VS<sub>fed</sub>); M is the methane yield at time t, (L/kg VS<sub>fed</sub>); k is the first order kinetic constant (d<sup>-1</sup>).

As the experimental data of cumulative methane yield with respect to time were available, upon considering the initial guess values for M<sub>u</sub> and k, fitting the non-linear equation through MS EXCEL (Microsoft Office 2013) software and solving the equation by Least Square Error (LSE) method in solver tool kit, the exact values of M<sub>u</sub> and k were obtained.

#### Modified Gompertz model

During biomethane production variables such as substrate concentration, microbial population and concentration of soluble metabolites were found to change periodically. Kinetic models such as modified logistic model, Richard model and Gompertz model have been proposed to describe such changes during the course of reaction [19]. Gompertz model has been extensively used to describe the progress of substrate degradation, bacterial growth and soluble metabolite production in a batch fermentative process [20]. As biogas production is assumed to be growth associated, the developed Gompertz equation has been modified to predict the methane production. This model was extensively used to simulate the methane production rate and its kinetic behaviour which has been reported elsewhere [21,22]. In the present investigation this model was applied for the co-digestion process from which kinetic parameters such as lag phase (λ, days), maximum methane production potential (L/kg VS<sub>fed</sub>) and maximal methane production rate (L/kg VS<sub>fed</sub>/d) were determined (Eq 3).

The equation employed for this study has been given below:

$$P = P_0 * \exp \left\{ -\exp \left[ \frac{R_m * e}{P_0} (\lambda - t) + 1 \right] \right\} \quad (3)$$

Where, P is the methane yield (L/kg VS<sub>fed</sub>) at given time during the digestion period; P<sub>0</sub> is the maximum methane production potential (L/kg VS<sub>fed</sub>); R<sub>m</sub> is the maximal methane production rate (L/kg VS<sub>fed</sub>/d). The value of e is equal to 2.7182.

### Unstructured segregated model

The non-linear equations that describe different stages of anaerobic digestion of wastes have been derived as given below:

#### Step 1: Hydrolysis of insoluble substrate

Formation of soluble substrate from insoluble complex macromolecules (hydrolysis) is assumed to be a first order reaction as represented (Eq 4).

$$\frac{dC_{is}}{dt} = -(K_{hs}) \times C_{is} \quad (4)$$

Where, dC<sub>is</sub>/dt denotes the rate of degradation of insoluble substrate; K<sub>hs</sub> is the hydrolytic constant (d<sup>-1</sup>) and C<sub>is</sub> represent concentration of insoluble substrate (g TS/L).

#### Step 2: Formation of soluble substrate (Eq. 5)

$$\frac{dC_s}{dt} = (a * K_{hs} * C_{is}) - \left( b * \left( \mu_{max-a} * \frac{C_{ss}}{K_{ss} + C_{ss}} \right) * X_a \right) \quad (5)$$

Where, a - Growth coefficient of hydrolytic microbes; b - Stoichiometric coefficient pertaining to growth of acidogenic microbes by utilizing soluble substrate (g cells formed/g soluble substrate utilized). Soluble substrates mainly constitute sugars, amino acids, VFA etc.; μ<sub>max-a</sub> - Maximum specific growth rate of acidogenic bacteria (d<sup>-1</sup>); C<sub>ss</sub> - Concentration of soluble substrate (g/L); K<sub>ss</sub> - Saturation constant of soluble substrate (mg/L); X<sub>a</sub> - Concentration of acidogenic bacteria (mg VS/L); dC<sub>s</sub>/dt - Rate of soluble substrate formation.

Eq. 5 governs the rate of formation of soluble substrate. First part of the equation denotes the rate of production of soluble substrate by the hydrolysis of insoluble substrate and the second part represents rate of consumption of soluble substrate by the acidogenic bacteria. Second part of the Eq. 5 was derived based on two equations (Eq. 6).

$$-\frac{ds}{dt} = \frac{1}{y(ss)} * \frac{dX_a}{dt} \quad (6)$$

Where, - $\frac{ds}{dt}$  - Rate of utilization at which soluble substrate;  $\frac{dX_a}{dt}$  - Rate at which acidogenic bacteria grows; y (ss) - Yield coefficient of acidogenic bacteria by utilizing the soluble substrate (g cells formed/g substrate utilized).

#### Step 3: Growth rate of acidogenic bacteria (Eq. 7)

Growth rate of acidogenic bacteria is governed by Eq. 7.

$$\frac{d(X_a)}{dt} = \left( \mu_{max-a} * \frac{C_{ss}}{K_{ss} + C_{ss}} \right) * X_a \quad (7)$$

The products obtained at the end of this step were assumed to be organic acids which act as the substrate for acetogenic bacteria to produce VFA.

#### Step 4: Formation rate of organic acid (Eq. 8).

The rate of formation of organic acid is expressed as given in Eq. 8.

$$\frac{dC_a}{dt} = \left( \left( c * \left( \mu_{max-a} * \frac{C_s}{K_{ss} + C_s} \right) * X_a \right) - \left( d * \left( \mu_{max-ac} * \frac{C_a}{K_a + C_a} \right) * X_{ac} \right) \right) \quad (8)$$

Where, c- Stoichiometric coefficient pertaining to formation of

organic acid by utilizing soluble substrate; d- Stoichiometric coefficient pertaining to growth of acetogenic microbes by utilizing organic acid (g of cell formed/g of substrate utilized) respectively; μ<sub>max-ac</sub> - Maximum specific growth rate of acetogenic bacteria (h<sup>-1</sup>); C<sub>a</sub> - Concentration of organic acid (mg/L);  $\frac{dC_a}{dt}$  - Rate of formation of organic acids; K<sub>a</sub> - Saturation constant with organic acids as substrate (mg/L); X<sub>ac</sub> - Concentration of acetogenic bacteria (mg VS/L). The first part of the Eq. 8 represents organic acid production rate and the second part denotes its utilization rate. The second part of the equation is formed using two equations (Eq. 9).

$$-\frac{ds}{dt} = \frac{1}{y(A)} * \frac{dX_{ac}}{dt} \quad (9)$$

Where, - $\frac{ds}{dt}$  - Rate of utilization of organic acids and  $\frac{dX_{ac}}{dt}$  - Growth rate of acetogenic bacteria; y(A) - Yield coefficient of acetogenic bacteria by utilizing the organic acids.

#### Step 5: Growth rate of acetogenic bacteria (Eq. 10)

$$\frac{d(X_{ac})}{dt} = \left( \mu_{max-ac} * \frac{C_a}{K_a + C_a} \right) * X_{ac} \quad (10)$$

Eq. 5 signifies the growth rate of acetogenic bacteria.

#### Step 6: Formation rate of VFA (Eq. 11)

The rate of formation of VFA is expressed as given in Eq. 11.

$$\frac{dVFA}{dt} = \left( \left( e * \left( \mu_{max-ac} * \frac{C_a}{K_a + C_a} \right) * X_{ac} \right) - \left( f * \left( \mu_{max-m} * \frac{C_{VFA}}{K_{VFA} + C_{VFA}} \right) * X_m \right) \right) \quad (11)$$

Where, e - Stoichiometric coefficient pertaining to formation of VFA by acetogenic microbes by utilizing organic acid; f - Stoichiometric coefficient pertaining to growth of methanogens by utilizing VFA; μ<sub>max-m</sub> - Maximum specific growth rate of methanogenic bacteria (h<sup>-1</sup>); C<sub>VFA</sub> - Concentration of VFA in terms of acetic acid (mg acetic acid /L); K<sub>VFA</sub> - Saturation constant with VFA as substrate (mg/L). The first part of the Eq. 11 represents VFA production rate and the second part is its utilization rate. The second part of the equation is formed using two equations (Eq. 12).

$$-\frac{ds}{dt} = \frac{1}{y(VFA)} * \frac{dX_m}{dt} \quad (12)$$

Where, - $\frac{ds}{dt}$  - Rate of VFA utilization;  $\frac{dX_m}{dt}$  - Growth rate of methanogenic bacteria; y (VFA) - Yield coefficient of methanogenic bacteria by utilizing the VFA.

#### Step 7: Growth rate of methanogenic bacteria (Eq. 13)

$$\frac{d(X_m)}{dt} = \left( \mu_{max-m} * \frac{C_{VFA}}{K_{VFA} + C_{VFA}} \right) * X_m \quad (13)$$

Eq.13 signifies the growth rate of methanogenic bacteria.

#### Step 8: Methane formation rate (Eq. 14)

The methane formation by methanogenic bacteria is a growth associated process. Hence, the rate of methane (product) formation is represented in terms of growth rate of methanogens as given in Eq. 14.

$$\frac{dCH_4}{dt} = \left( g * \left( \left( \mu_{max-m} * \frac{C_{VFA}}{K_{VFA} + C_{VFA}} \right) * X_m \right) \right) \quad (14)$$

Where, g - Stoichiometric constant pertaining to formation of methane from methanogens (Eq. 15).

The above equation (Eq. 14) was derived using Eq. 15 as given below,

$$\frac{dp}{dt} = \frac{1}{y(ps)} * \mu_{max-m} * \frac{dX_m}{dt} \quad (15)$$

Where,  $\frac{dp}{dt}$  represents the methane formation rate and  $\frac{dX_m}{dt}$  is the growth rate of methanogenic bacteria;  $y(ps)$  is the yield coefficient of methane formation by utilizing the VFA.

The derived model is classified as unstructured because the microbial metabolism for its cellular maintenance is not taken into account and denoted as segregated since different group of microorganisms namely hydrolytic, acidogenic, acetogenic and methanogenic bacteria were considered. This model consist of kinetic coefficients/constants such as  $a, b, c, d, e$  and kinetic parameters  $K_{hs}, K_{ss}, K_{VFA}, \mu_{max-a}, \mu_{max-m}$ . MATLAB version (2012b) (Math Works Inc., USA) has been employed using the built-in optimisation routine based on Levenberg-Marquardt method. The equations were integrated using ordinary differential equation (ODE) solver by Gear's algorithm. The initial conditions for the set of ODE consist of initial concentration of substrate and inoculum. All the constants of the model equations were taken as the model parameters. For unstructured segregated kinetic modeling the experimental data

obtained from CCD run order were used and the obtained simulated output of methane profile was used for plotting the correlation plot. The flow chart for performing the kinetic model in MATLAB has been provided in Figure 1.

### Theoretical considerations of the model

a. The influent substrate ( $C_{is}$ ) mainly constitute of unhydrolyzed insoluble organic components. Upon action of hydrolytic bacteria these complex organic compounds were converted to soluble substrate ( $C_{ss}$ ) such as sugar, amino acids, alcohol, fatty acid, etc., that can be easily assimilated by the microorganisms.

b. The solubilized organic components were consumed by the acidogenic microorganisms for its growth and product formation. The end products of the assimilation were considered to be mainly of organic acids ( $C_a$ ) such as propionic acid, butyric acid etc. These organic acids were converted to VFA ( $C_{VFA}$ ) by the action of acetogenic microorganisms.

c. VFAs were further utilized by the methanogenic microorganisms. The resultant end products were methane and carbon di-oxide.

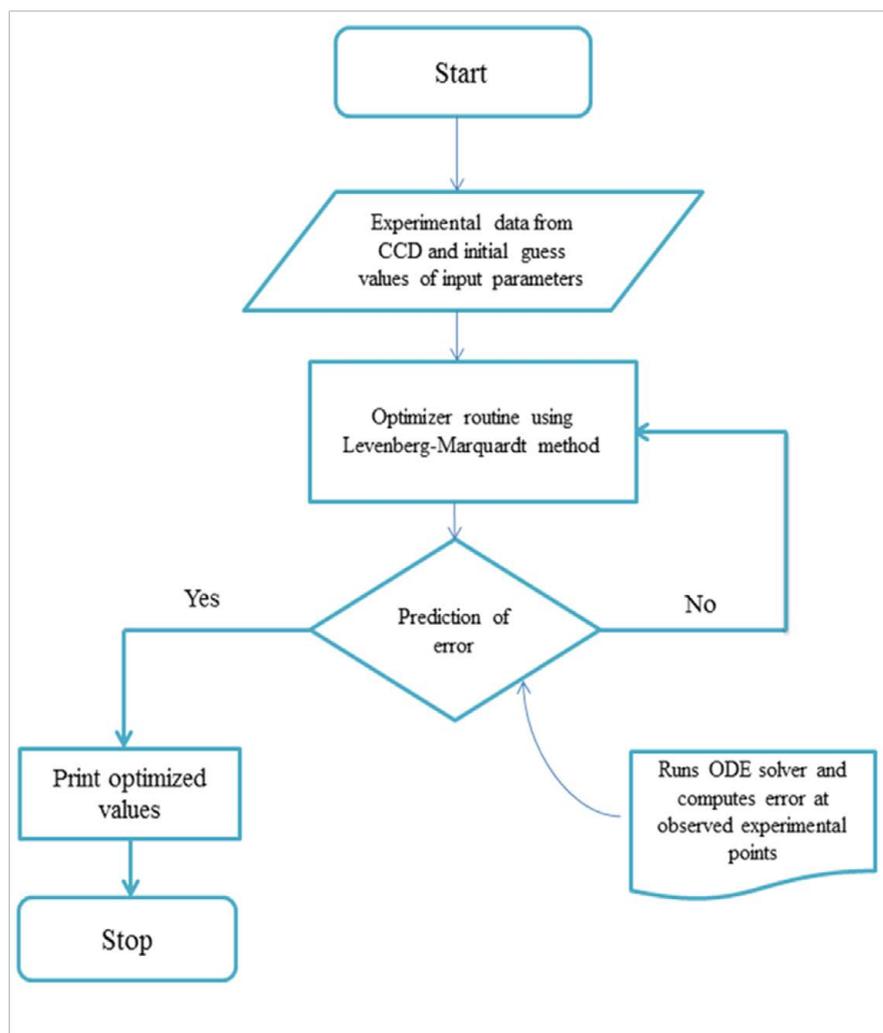


Figure 1: Flow chart of the program used to run the unstructured segregated model in MATLAB.

d. The overall metabolic reactions of this process were carried out by the synergistic action of different trophs of microorganisms such as fermentative, acidogenic, acetogenic and methanogenic. Therefore, kinetic constants of the model would be proposed as global.

e. The digester is assumed to be a completely mixed reactor.

## Results and Discussion

### Optimization of anaerobic co-digestion

The experimental methane yield obtained from CCD has been represented in Table 3. Significance of the model was analyzed through ANOVA as tabulated in Table 4. From the Table 4, it has been observed that higher F-value (186.65) and lower p-value (<0.0001) of the model show the significance of the regression model.

The second order polynomial regression model equation obtained for VW+PS in coded form is represented in Eq. 16.

Methane yield (L/kg VS<sub>fed</sub>) = 560.56 + 78.14 A + 13.35B + 6.413C - 7.33 A<sup>2</sup> - 0.119 B<sup>2</sup> - 0.091C<sup>2</sup> + 0.436 AB - 0.074AC + 0.025 BC (Eq. 16).

From the Eq. 16, it has been observed that the chosen parameters were found to be significant and positively influence the methane yield. Further, upon analyzing the effect of individual parameters and their significance, it has been perceived that A, B, C, A<sup>2</sup>, B<sup>2</sup>, C<sup>2</sup>, AC and BC were found to have lower p value (p<0.05) which signifies that these parameters were significant towards the output (Methane yield). Regression coefficient (R<sup>2</sup>) of 0.9641 signifies that model could explain the 96.41% of process behavior and only 3.59% variation could not explained by the model.

### Response surface plots (Figure 2)

From the Figure 2A, it has been observed that there was a significant correlation between substrate and co-substrate concentration. The biomethane yield was found to increase from 5 g TS/L (260 L/kg VS<sub>fed</sub>)

to 7.5 g TS/ L (~350 L/kg VS<sub>fed</sub>) after that there was decline in the yield. Similarly, when the proportion of PS was increased from 20 to 40% (TS, w/w) there was an increasing trend with enhancement of methane yield from 258 L/kg VS<sub>fed</sub> to 354 L/kg VS<sub>fed</sub> after that, a reduction in yield was observed.

Figure 2B represents the interactive effect of substrate and inoculum concentration. It has been observed that there was an increase in methane yield which is in parallel with increase in inoculum concentration. After 75% (VS/VS) (~335 L/kg VS<sub>fed</sub>) there was a reduction in methane yield with further increase in substrate concentration.

In case of interaction between inoculum concentration and co-substrate proportion, there was a direct proportionality between these two factors. As depicted in Figure 2C, when the concentration of inoculum gradually increased from the initial value of 60% (VS/VS) the corresponding methane yield also found to increase reaching a maxima at 75% (VS/VS) (~340 L/kg VS<sub>fed</sub>) whereas in the respective co-substrate proportion there was a hyperbolic pattern of increase in methane yield with the increase in proportion followed by a drastic reduction in yield beyond 40% (TS, w/w) reaching a minimum of 262 L/kg VS<sub>fed</sub> at 60% (TS, w/w).

When there is increased loading of PS (i.e., >40% TS w/w) there is possibility of ammonia inhibition due to the breakdown and conversion of organic nitrogen present in it. This might be the plausible reason for sudden drop in methane yield when co-substrate addition was increased above 40% (TS, w/w).

### Validation of the model

The data obtained from the CCD model was fitted with the actual experimental data and found to have a good correlation (R<sup>2</sup>-0.9641). The optimized parameters obtained from the response optimizer function of the software interface were substrate loading of 7.37 g TS/L in which PS constitute 42.62% (w/w) and inoculum concentration of 74.08% (VS/VS) which resulted in predicted methane yield of 359.26 L/kg VS<sub>fed</sub>. The outcome of validation experiment was found to be 353.41 ± 15.45 L/kg VS<sub>fed</sub>. The standard error obtained between the experimental and predicted yield was found to be (±) 1.62% which signifies that developed model could predict the process behavior within the minimal error.

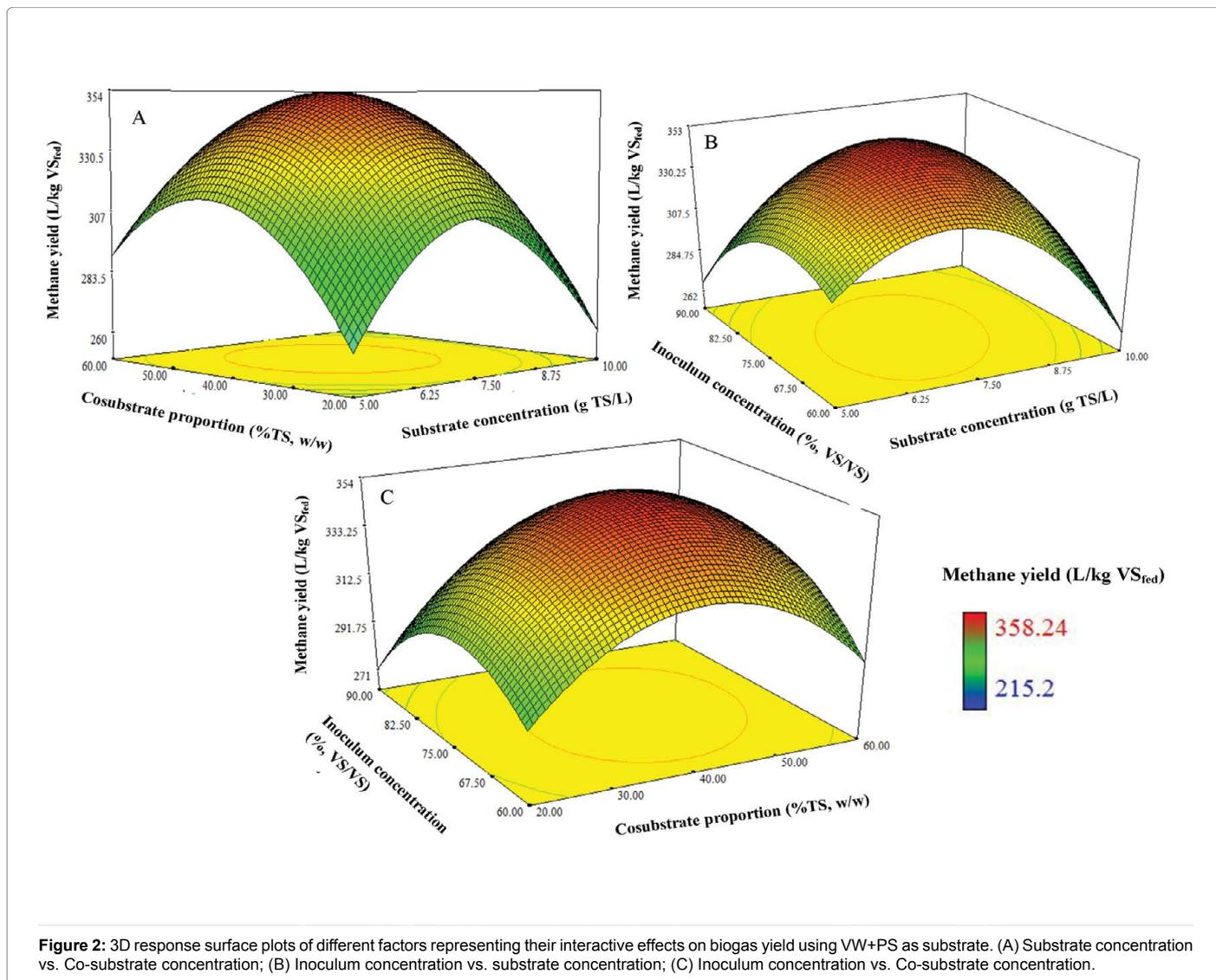
It has been reported that anaerobic digestion of VW yielded maximum methane yield in the range of 230-450 L/kg VS<sub>fed</sub> [23]. The results obtained from this study (353.41 ± 15.45L/kg VS<sub>fed</sub>) also lie in that range but the significance involves in utilization of MAC as an inoculum. Usually sources like animal manure, digested sludge, etc., have been used as the conventional inoculum for treatment of VW, however, for large scale implementation of biogas plants the availability of huge amount of inoculum and its transportation may not be feasible at times, that limits the viable option of waste treatment. Thus the outcome of the present study revealed that MAC could serve as a substitute for conventional inoculum sources which could mitigate the bottleneck associated during scale up operations.

### Kinetic modeling

**First order kinetic model fit:** Figure 3A represents the first order kinetic model fit of the anaerobic co-digestion process with R<sup>2</sup> of 0.9914 (Table 5). The obtained kinetic parameters have been tabulated in Table 5. Simplified generalized models based on first order kinetic equation have been used for designing and sizing of continuous stirred tank and batch reactors respectively [24,25]. All the reported study insisted that

Run order	Substrate concentration (A) (g TS/L)	Inoculum concentration (B) (% VS/VS)	Cosubstrate proportion (C) (% TS, w/w)	Methane yield (L/kg VS <sub>fed</sub> ) Experimental
1	7.5	75	40	354.33 ± 17.5
2	10.0	60	60	222.45 ± 11.5
3	10.0	90	60	271.76 ± 13.0
4	7.5	75	40	354.41 ± 18.1
5	7.5	75	20	247.32 ± 12.4
6	7.5	75	40	361.11±17.8
7	10.0	90	20	248.63 ± 12.6
8	7.5	75	40	353.28 ± 17.7
9	5.0	90	20	215.21 ± 10.8
10	7.5	60	40	325.39 ± 16.3
11	5.0	75	40	289.44 ± 14.5
12	5.0	90	60	241.62 ± 12.1
13	7.5	90	60	344.71 ± 17.2
14	10.0	60	20	262.91 ± 13.1
15	7.5	75	40	358.32 ± 17.9
16	5.0	60	20	267.37 ± 13.4
17	7.5	90	40	320.43 ± 16.0
18	5.0	60	60	287.63 ± 14.4
19	7.5	75	40	358.21 ± 17.9
20	10.0	75	40	296.28 ± 14.1

**Table 3:** CCD experimental design with the corresponding observed methane yield obtained from VW+PS co-digestion.



Source	Sum of Squares	df	Mean Square	F value	p-value
Model	47812.4	9	5312.5	186.65	< 0.0001
A-Substrate conc.	1194.3	1	2269.6	79.74	0.0012
B-Inoculum conc.	791.4	1	1054	37.03	<0.0001
C-Co-substrate conc.	301.6	1	1619	56.85	<0.0001
A <sup>2</sup>	35061.1	1	5782.7	203.17	<0.0001
B <sup>2</sup>	6894.9	1	3640.9	127.92	<0.0001
C <sup>2</sup>	1968	1	1968	69.15	<0.0001
AB	110.7	1	110.7	3.89	0.077
BC	446.1	1	446.1	15.67	0.003
AC	2137.3	1	2137.3	75.09	<0.0001
Residual Error	5043.7	10	504.5		
Lack of fit	4992.5	5	998.5	3.74	0.54
Pure error	51.2	5	10.2		

R<sup>2</sup>– 0.9641; R<sup>2</sup>(pred) – 0.9331; R<sup>2</sup>(adj) – 0.988

**Table 4:** ANOVA analysis of CCD based RSM for VW+PS co-digestion.

the substrate degradation and subsequent biogas production follows the first order kinetics. First order model include a kinetic constant 'k'

Model	Kinetic parameters	Values
First order	Maximum methane yield ( $M_u$ , L/kg VS <sub>fed</sub> )	421
	k (d <sup>-1</sup> )	0.0490
	R <sup>2</sup>	0.9914
Modified Gompertz	Methane production potential (P) (L/kg VS <sub>fed</sub> )	374.09
	Maximal methane production potential (R) (L/kg VS <sub>fed</sub> /d)	17.269
	Lag phase ( $\lambda$ ) (days)	3.935
	R <sup>2</sup>	0.9993
	Experimental methane yield (M, L/kgVS <sub>fed</sub> )	353.41 ± 15.45

**Table 5:** Kinetic parameters obtained from model fit.

which denotes the rate of biogas production. More positive inclination of k denotes faster rate of biogas production [26]. In the present study, the value of k was found to be 0.049 d<sup>-1</sup> which is similar to the reported range of 0.017-0.04 d<sup>-1</sup> [25]. It has been observed that the maximum methane yield ( $M_u$ ) was observed to be 421 L/kg VS<sub>fed</sub> which was an over prediction than that of the observed yield (353.41 ± 15.45 L/

kg VS<sub>fed</sub>). The predicted maximum methane yield is the resultant of complete degradation of all the biodegradable components of the substrate which may take an indefinite period in actual condition and thus it is obvious for the lesser observed methane yield. Simple first order kinetics provide moderately good results because it is suited for the homogeneous reaction mixture whereas, in the present case the substrates (VW and PS) are heterogeneous consisting of both readily and slow degradable organic substances. Thus a more flexible and widely adaptable Gompertz model was employed to get an insight into the kinetic behaviour of the process.

**Modified Gompertz model fit:** The modified Gompertz equation suits for the heterogeneous substrate nature which relates cumulative methane production, methane yield potential, the maximum production rate and the duration of lag phase with an assumption that methane produced is a function of bacterial growth in batch digesters. As represented in Figure 3B, the modified Gompertz model fitted well with the experimental methane production data obtained from the validation experiment with R<sup>2</sup> (coefficient of determination) ~0.99. The kinetic parameters obtained from this model have been represented in Table 5. The lag phase of the digestion process was observed to

be 3.9 days. Substrates having high fibres, cellulose, etc., showed a distinct lag phase of 1-4 days that corresponds to the period required for hydrolysis of long chain branched polymers prior to fermentation and methanogenesis initiation [27]. The maximum methane yield obtained from the model was found to match well with the methane yield obtained from the validation experiment. Maximum methane production potential was observed to be 17.27 L/kg VS<sub>fed</sub>/d. Modified Gompertz equation have been applied by many other researchers for estimating biomethane production potential and lag phase of the process using different organic wastes as substrates. The sigmoid pattern obtained from the present study is similar to the results reported in the literature [21,28,29].

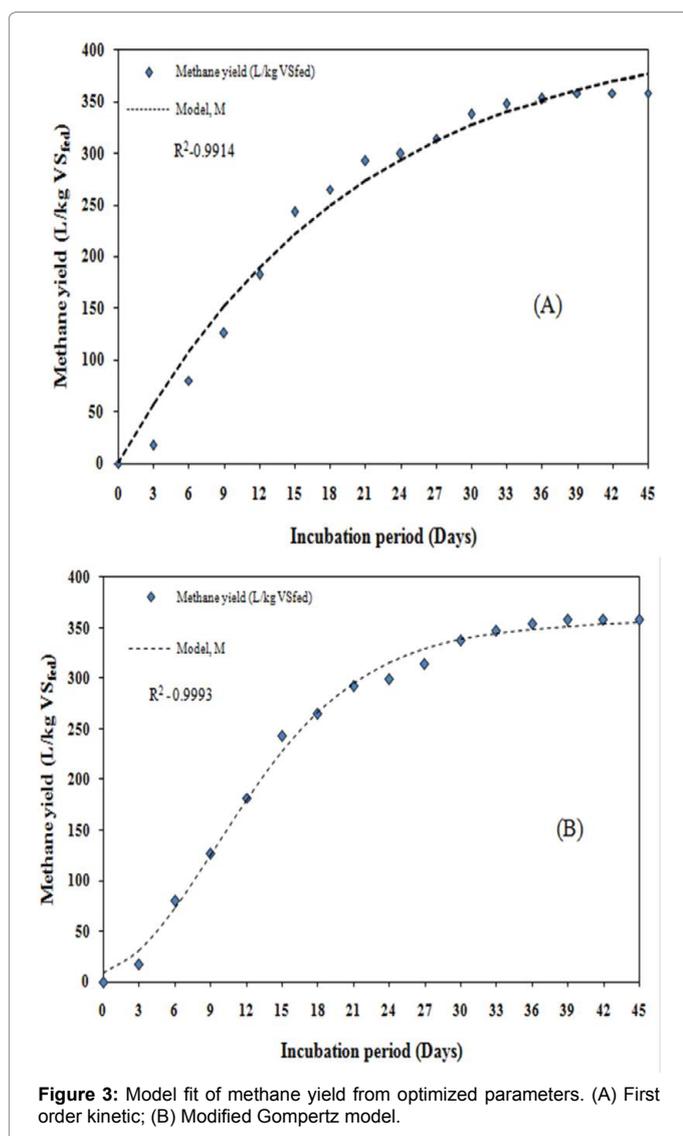
**Unstructured segregated model:** Unstructured segregated model developed based on the Monod microbial growth kinetics is a unique model which has been developed for this co-digestion study. Garcia-Ochoa et al. and Husain have described kinetics of the anaerobic digestion process based on the Monod equation [30,31]. An unstructured non-segregated model was derived based on the Monod and Contois kinetic equations to study the effect of process parameters such as temperature and influent substrate concentration on specific growth rate of microorganism ( $\mu$ ) and microbial adhesion to the substrate (K) [32,33]. Similarly to represent the dynamic behaviour of the anaerobic digestion process using animal wastes as substrate, an unstructured segregated kinetic model based on Monod equation was reported which included six different equations and ten kinetic parameters [10,34].

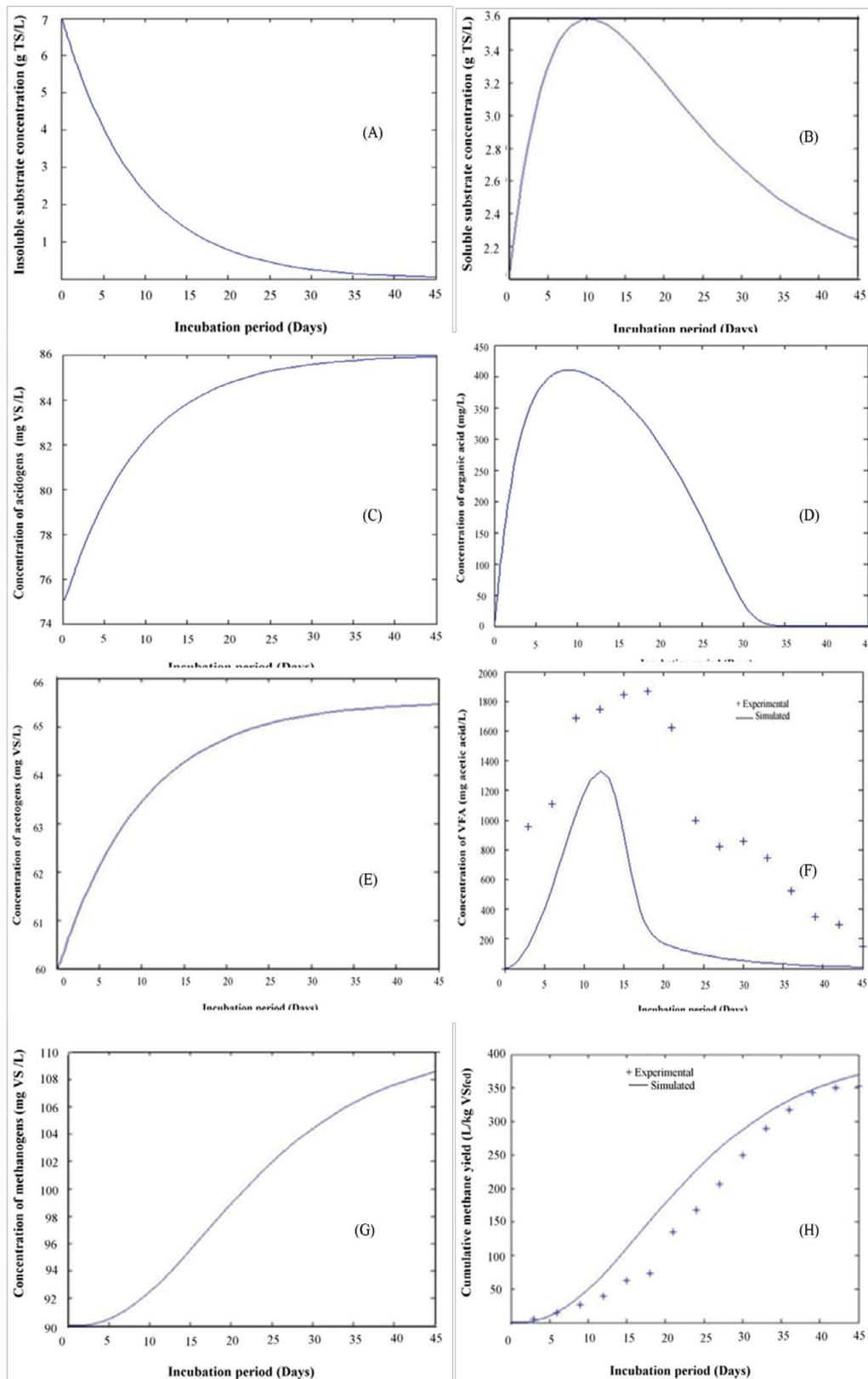
**In the present study, four main stages have been considered to model the anaerobic process:**

- (1) Hydrolysis of the insoluble substrate.
- (2) Acidogenesis by acidogenic bacteria through uptake of solubilised substrate to produce acidified substrate for acetogenic/methanogenic microbes.
- (3) Uptake of acidified substrate by acetogenic bacteria for growth and formation of VFA.
- (4) Assimilation of VFA for growth of methanogenic bacteria and methane production (Figures 4A-4H).

The kinetic plots obtained while solving the non-linear equations of unstructured segregated model have been represented in Figures 4A-4H. Hydrolysis is the rate limiting step in most of the anaerobic digestion process with certain exceptions where methanogenesis is reported to be limiting [35]. Hydrolysis of particulate substrate into simpler molecules aids in easy transport into the cell for its further metabolism. Different mechanism on hydrolysis process has been reported in the literature which include microbial enzymes mediated hydrolysis of complex substrate and adherence of microbial cells over the surface of the substrate and release of enzyme for production of soluble organic molecules from the insoluble particulate macromolecules [36,37].

In the present study as denoted in Eq. 4, most conventional model to simulate the hydrolysis which involves first order rate law has been utilized for determination of hydrolysis constant. Figure 4A, represents the pattern of hydrolysis of the insoluble substrate during the digestion process. It has been observed that insoluble substrate degradation was found to initiate from day-1 of incubation followed by a steep decline with time. Practically, it is not possible for microorganisms to completely degrade all the insoluble substrates. This has been denoted by a non-zero decreasing trend in the substrate hydrolysis profile Figure 4A. The hydrolysis constant obtained in this study





**Figure 4:** Kinetic profiles obtained from the unstructured segregated model (VW+PS). (A) Insoluble substrate concentration; (B) Soluble substrate concentration; (C) Acidogenic biomass profile; (D) Organic acid profile; (E) Acetogenic biomass profile; (F) VFA profile (experimental and simulated); (G) Methanogenic biomass profile; (H) Methane profile (experimental and simulated).

was found to be 0.1 d<sup>-1</sup>. During VW digestion hydrolysis constant was reported to be 0.34 d<sup>-1</sup> [37]. However, in a study conducted with crop and crop residues the hydrolysis constant was found to range between 0.009-0.094 d<sup>-1</sup> which is similar to the outcome of the present study where VW was co-digested with aquatic weed PS [38].

Followed by hydrolysis, acidogenic phase of the process takes place. It has been reported that bacteria that participate in hydrolysis of polymeric substrates are also active in acidogenic phase of anaerobic digestion [39]. Thus the hydrolytic and acidogenic bacteria are collectively termed as fermentative bacteria. These bacteria can be either facultative or obligate. Most of the Enterobacteriaceae are active fermentative microbes that involve in breakdown of complex biomolecules [40]. As represented in Figure 4B, a bell shaped curve was obtained for soluble substrate concentration profile. Maximum soluble substrate of 3.6 g TS/L was obtained in 10 days of incubation which is in parallel to the hydrolysis profile of insoluble substrate.

Acidogenesis is a microbial process that includes degradation of soluble sugars and amino acids into simpler products such as organic acids. This is the fastest step of the anaerobic conversion of complex organic matter. Thus, changes in acidogenic rate constants do not influence the methane production rate [35]. However, acetic acid which is the major precursor for methane formation is produced through acetogenesis pathway where in which the organic acids serve as substrate for acetogenic bacteria. Figure 4C depicts the changes in acidogenic population during digestion of VW+PS. It has been seen that the profile followed 'L' shaped pattern where there is no distinct adaptation period for acidogenic bacteria. This phenomenon might be due to progressive increase in soluble substrate concentration from day-1 to day-10 which enables the availability of hydrolytic products that could be metabolized by the acidogens for its conversion into organic acids. Attainment of maximum organic acid matched with the profile of solubilized substrate formation profile where maximum concentration of 3.6 g/L was achieved (Figure 4D).

Pavlostathis and Giraldo-Gomez reported kinetic constants of anaerobic oxidation of fatty acid where the values of saturation constant ( $K_s$ ),  $\mu_m$  and yield coefficient ranged between 12-3180 mg COD/L, 0.13-1.2 d<sup>-1</sup> and 0.01-0.047 mg/mg respectively [41] (Table 6). In the present study as depicted from Table 6, the value of saturation constant ( $K_a$ ) was found to be 36.41 mg/L. In case of growth kinetic behaviour of acidogens,  $\mu_m$  was found to be 1.85 d<sup>-1</sup> whereas, the growth

coefficient was observed to be 2.5 mg/g. It is worth mentioning that obtained kinetic constants were found to match with the reported range which suggests the adequacy of the developed model equation for the acidogenic step.

Followed by acidogenic step, acetogenesis process takes place. Mostly acetic acid acts as a precursor for methane formation which constitutes approximately 70% of total methane production [42]. VFA present in the digester is metabolized by the acetoclastic methanogens into carbon di-oxide and methane [43]. Since methanogens are sensitive to fluctuation in the physiological pH, when the population of acetogens out-number the methanogens there is a possibility of feedback inhibition by the undissociated acetic acid which results in the drop in pH that lead to process failure. However, no considerable inhibition to methanogens was observed at acetate concentration of 2400 mg/L in a study conducted by Wang et al. which is differing with previous reports where concentration of VFAs above 2000 mg/L has been reported to inhibit methane production [44,45]. From the Table 6, the saturation constant ( $K_{VFA}$ ) 881.7 mg/L was found to lie in the reported range of  $K_s$  values of acetic acid 154-869 mg/L [46].

It has been observed that the acetogenic microbial population showed a characteristic sigmoidal growth pattern (Figure 4E) which is similar to the results of the kinetic study reported with livestock manure as substrate [30]. The VFA profile measured in terms of mg acetic acid/L during the course of digestion was found to match well with the simulated profile (Figure 4F). From the growth curve of acetogenic biomass, it can be deciphered that stationary phase was achieved between 15-20 days. During this the exponential growth period, i.e., within 10 days there was maximum production of VFA (~1600 mg/L) after that there was a fall in the concentration (Figure 4F) which might be due to the utilization of VFA by methanogens for conversion to methane and carbon di-oxide.

The increase in VFA concentration and reaching maximum of ~1600 mg acetic acid/L is in concomitant with the acetogens growth profile where the maximum VFA concentration was achieved during the log phase of the acetogenic population (Figure 4G). In addition, the decreasing trend of soluble substrate concentration and increase in VFA concentration during the digestion period of 10-25 days illustrates the possible utilization of hydrolyzed products by the acido/acetogens for production of VFA. During biogas production there was lag phase upto 5 days and exponential biogas production upto 30 days (Figure 4H) which is in concomitant with the decrease in VFA concentration in the reaction medium (Figure 4F).

VFA produced during the acetogenesis stage of digestion process serves as the substrate for methanogenic counterpart for production of methane. This syntrophic association between these two microbial populations is well represented by the methanogenic growth profile (Figure 4G) and methane production pattern (Figure 4H). The methanogenic population profile was found to follow a sigmoid growth pattern. As methane production from the methanogens is a growth associated process the pattern of methane yield is similar to the growth of methanogens as represented in Figures 4G and 4H. From the predicted and observed values of VFA and methane Figures 4F and 4H, it has been seen that the model equation predicts the behaviour of the system almost near to the real time situation. The yield coefficient of anaerobic biomass usually lies between 0.05-0.2 g of biomass/g of substrate consumed whereas the aerobic biomass could be as high as 0.5 g of biomass/g of substrate consumed [47]. In the present study, the yield coefficient of acetogens and methanogenic biomass were found to be 990 mg/g and 50 mg/g respectively. The yield coefficient of hydrolytic

Kinetic constants	Unit	KW+PS
a	g/g	0.400
b	mg/g	5.700
c	mg/g	1.540
d	mg/g	2.500
e	mg/g	990.000
f	mg/g	50.000
g	-	00.990
$K_{hs}$	d <sup>-1</sup>	00.100
$K_{ss}$	mg/L	14.120
$K_a$	mg/L	36.410
$K_{VFA}$	mg/L	881.680
$\mu_{max-a}$	d <sup>-1</sup>	01.850
$\mu_{max-ac}$	h <sup>-1</sup>	00.075
$\mu_{max-m}$	h <sup>-1</sup>	00.067

**Table 6:** Kinetic constants and stoichiometric coefficients of unstructured segregated model.

Anaerobic digestion step	Kinetic equation from Unstructured segregated model
Hydrolysis of insoluble substrate	$\frac{dC_s}{dt} = (0.4 * 0.0042 * C_{is}) - \left( 5.7 * \left( 0.077 * \frac{C_{ss}}{14.12 + C_{ss}} \right) * X_a \right)$
Formation of soluble substrate	$\frac{dC_s}{dt} = (0.4 * 0.0042 * C_{is}) - \left( 5.7 * \left( 0.077 * \frac{C_{ss}}{14.12 + C_{ss}} \right) * X_a \right)$
Formation rate of organic acid	$\frac{dC_a}{dt} = \left( \left( 1.54 * \left( 1.85 * \frac{C_s}{14.12 + C_s} \right) * X_a \right) - \left( 2.5 * \left( 0.075 * \frac{C_a}{36.41 + C_a} \right) * X_{ac} \right) \right)$
Formation rate of VFA	$\left( \left( 0.99 * \left( 0.075 * \frac{C_a}{36.41 + C_a} \right) * X_{ac} \right) - \left( 0.05 * \left( 0.067 * \frac{C_{VFA}}{881.7 + C_{VFA}} \right) * X_m \right) \right)$
Methane formation rate	$\frac{dCH_4}{dt} = \left( 0.99 * \left( \left( 0.067 * \frac{C_{VFA}}{881.7 + C_{VFA}} \right) * X_m \right) \right)$

VFA: Volatile Fatty Acids

Table 7: Kinetic equations of each step of anaerobic co-digestion process.

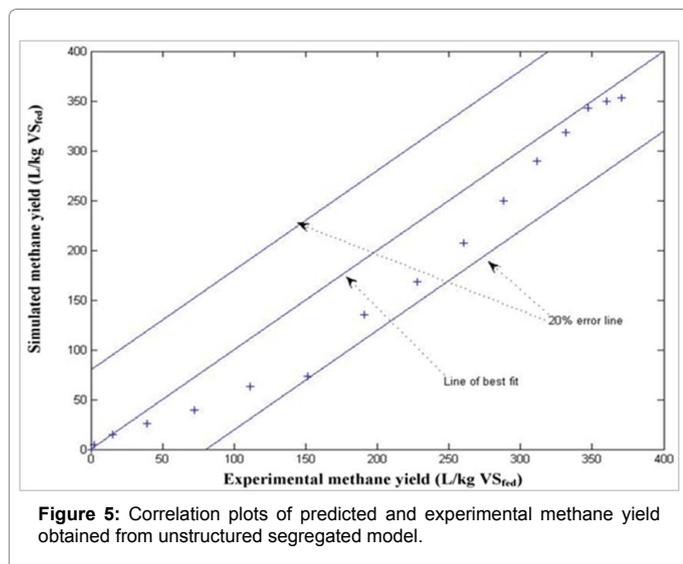


Figure 5: Correlation plots of predicted and experimental methane yield obtained from unstructured segregated model.

bacteria was found to be 0.4 g/g. In addition to it, the maximum specific growth rate of acetogenic and methanogenic biomass was found to be 0.075 and 0.067 h<sup>-1</sup>, respectively. Archer and Powell investigated the dependence of specific growth rate of methanogenic mutualistic co-culture on the methanogenesis and reported that μ<sub>m</sub> of methanogenic microorganisms varied from 0.04-0.17 h<sup>-1</sup> [48]. Methanogens are characterized by growing under wide range of temperature. However, well established optima exist around 35-40°C for mesophilic and 55°C for thermophilic [49]. Although thermophilic system contribute 25-50% higher biogas yield than mesophilic, thermophilic conditions are more susceptible to variation in environmental factors and likely to inhibit the process due to VFA accumulation [27]. Thus mesophilic temperature range is best suited for full scale operation and because of this reason in the present study mesophilic temperature of 37°C is fixed as an optimal condition of co-digestion process.

From the obtained kinetic graphs, it has been seen that steps involved in the process (hydrolysis, acidogenesis, acetogenesis and methanogenesis) were carried out in a serial way. The hydrolytic step was found to proceed throughout the digestion period as there was a

need for soluble substrates for acidogenic, acetogenic and methanogenic microorganisms. The aforementioned serial process supports the proposed 4 step mechanism of hydrolysis-acidogenesis-acetogenesis-methanogenesis using the unstructured segregated model. The kinetic equations of each step of anaerobic co-digestion process with obtained kinetic constants through LSE method have been represented (Table 7).

Based on the obtained kinetic parameters, the validation of the model was assessed through the methane correlation plots (Figure 5). It can be seen from the Figure 5, the experimental methane yield profile fit well within ± 20% error band. It has been suggested that correlation coefficient should be at least 0.80 for the good fit of a model [50]. Simulated results for biomethane yield are within the reasonable limits thereby showing that the model presented a good stability and predictability. The kinetic parameters obtained represent the efficacy of different microorganisms influencing the anaerobic digestion. With the number of parameters and processes considered, the developed model outlined in the present study could effectively describe the dynamic features of the anaerobic co-digestion of VW and PS.

## Conclusion

Optimization of the process parameters revealed that PS added as a co-substrate positively influenced the biomethanation process. Three different models have been examined for analyzing the kinetics of the anaerobic digestion. The established kinetic models such as first order and modified Gompertz model fit well with the observed process behavior from VW+PS co-digestion. An unstructured segregated model was developed for probing the kinetic behavior of anaerobic co-digestion with mixed anaerobic culture as an inoculum which is a novel attempt of the present study. The developed model was found to predict the process dynamics within the acceptable error limit. The kinetic constants and equations obtained from the model will be highly helpful in scale up and large scale implementation of biogas plants where risk factors can be considerably reduced. Subsequent refinements in the model with the addition of VFA inhibition and temperature kinetics can further assist in development of design and operational strategies for improved performance.

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