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**Development of Simplified Anaerobic Digestion Models (SADM's) for studying
Anaerobic Biodegradability and Kinetics of Complex Biomass**

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ABSTRACT

The anaerobic co-digestion of cow manure and waste paper at ambient temperature condition was observed to be optimized at a mix proportion of 75:25 respectively. The development and testing of a set of simplified anaerobic digestion models (SADM's) for this mixture revealed that the Hill's based biogas yield rate model was most appropriate in describing the kinetics of biogas production. Parameter estimation using non-linear regression revealed that the half saturation constants expressed as acidified substrate and volatile solids equivalents were 0.228g/L and 5.340g VS/L respectively, and the maximum specific biogas yield rate and biodegradability were 2.2mL/g VS/day and 0.313 respectively. The coefficients "n" and "m" indicative of acidogenic bacterial adaptation for degradation and acetogenic/methanogenic bacterial cooperativity were estimated to be 1.360 and 2.738 respectively, while hydrolysis/acidogenesis was considered the rate limiting step. The need of bacterial adaptation may be an important factor to consider during anaerobic modeling of complex biomass.

Keywords: Anaerobic process; biodegradability; biogas; kinetic parameters; growth kinetics; rate limiting

Abbreviations

A_f = rate limiting step coefficient for fast substrate utilization

A_s = rate limiting step coefficient for very slow substrate utilization

$A_{f(s)}$ = rate limiting step coefficient for fast or very slow substrate utilization

b = fraction of initial volatile solids remaining in effluent

k_s = Monod's half saturation constant for acidified substrate (g/L)

K_s = Monod's half saturation constants in volatile solids equivalents (g/L)

k_n = Hill's half saturation constant for acidified substrate (g/L)

K_n = Hill's half saturation constant in volatile solids equivalents (g/L)

k_i = substrate inhibition constant for acidified substrate (g/L)

m = coefficient of acetogenic/methanogenic bacteria adaptation for cooperativity

n = coefficient of acidogenic bacteria adaptation for complex substrate degradation

R_f = recalcitrant fraction

R_{max} = maximum specific biogas yield rate (mL/g VS/day)

R = specific biogas yield rate (mL/g VS /day)

S_0 = initial volatile solids concentration (g/L)

S = volatile solids concentration remaining (g/L)

$X_{(a)}$ = acidogenic biomass concentration (mass/volume)

$X_{(a/m)}$ = acetogenic/methanogenic biomass concentration (mass/volume)

S_h = concentration of acidified substrate generated (g/L)

$S_{h(i)}$ = concentration of acidified substrate remaining intracellularly

$S_{h(u)}$ = concentration of utilized acidified substrate by the acetogenic/methanogenic biomass (g/L)

μ = bacteria growth rate (/day)

$\mu_{\max (a)}$ = maximum acidogenic bacteria growth rate (/day)

$\mu_{\max (a/m)}$ = maximum acetogenic/methanogenic bacteria growth rate (/day)

$Y_{x/s (a)}$ = yield coefficient for acidogenic biomass production (g/L)/ (g VS/L)

$Y_{x/s (a/m)}$ = yield coefficient for acetogenic/methanogenic biomass production (g/L)/ (g VS/L)

$Y_{y/s}$ = yield coefficient for biogas production (mL/g VS)/(g VS/L)

$K_{H(a)}$ = maximum substrate utilization rate by acidogenic bacteria (g VS_{utilized}/L/day)

$K_{H (a/m)}$ = maximum substrate utilization rate by acetogenic/methanogenic bacteria
(g acidified substrate_{utilized}/L/day)

y_t = biogas yield (mL/ g VS)

t = time (day)

1. Introduction

Anaerobic processes in waste management have been widely applied on account of their operational simplicity and potential of energy recovery [1]. Anaerobic digestion is the breakdown of organic material to produce biogas which is a mixture of methane and carbondioxide that is catalyzed by a consortium of micro-organisms in a series of interlinked biochemical reactions. These biochemical reactions comprise of hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrolysis is the breakdown of complex biomass into monomeric units; acidogenesis is the conversion of the monomers into volatile fatty acids; acetogenesis is the conversion of the volatile fatty acids into acetic acid and methanogenesis is the conversion of acetic acid into methane and carbondioxide [2, 3]. Anaerobic digestion has been identified as not only a viable means of producing carbon neutral energy [4] but also a means of mitigating the adverse effect of uncontrolled greenhouse gas emissions during decay of organic matter in the environment [5].

Organic substrate utilized for anaerobic digestion range from wastewater to complex organic feed stock such as animal manure, agricultural and industrial waste [6]. However, more recently, the process of co-digesting complex feedstock has been reported to result in improved biogas yield [7, 8]. Thus, for proper utilization of raw material in anaerobic digestion, adequate understanding of anaerobic biodegradation kinetics is imperative. Although, the anaerobic biodegradation kinetics of wastewater is well established, it has been poorly developed for complex biomass due to various reasons.

Most of the models used for studying biodegradation kinetics are based on maximum specific growth rate ($\mu_{\max}$) which requires short retention time that is not feasible for the complex biomass. In addition, the differentiation between bacteria volatile suspended solids and complex biomass volatile solids can be very difficult [9, 10]. Also, most of the models currently in use are based on a soluble growth limiting substrate whereas complex biomass exists in non-soluble form [11], in addition, the presence of recalcitrant fractions in complex biomass can render some of the volatile solids unavailable for bacteria, thus providing a false measure of the available substrate [11].

Most of the earlier available models used for anaerobic digestion did not account for the complex nature of the natural feedstock material because the substrate was assumed to be homogenous and biodegradable. However, in situations where models attempt to account for the nature of complex feedstock, they were restricted to particular substrate such as liquid manure [12, 13], sewage sludge [14] or biological waste [15].

Recently, the utilization of advance models which require extensive characterization of feedstock utilized in anaerobic digestion has dominated literature. The anaerobic digestion model No. 1 (ADM 1) [2] and the model developed by Angelidaki et al. [16] are examples of generalized models used for

studying anaerobic digestion of complex and co-digested biomass respectively. These models are rigorous and require large input parameters. Also, they are the most appropriate tool for studying the operation and technology development of anaerobic process [1, 17].

For designing of anaerobic processes, simplified model are considered most appropriate [18, 1]. Although, the need for a two stage model comprising hydrolysis and uptake of hydrolyzed substrate has been viewed by Batstone [1] as more appropriate for designing anaerobic processes, simplified generalized models based on first order models involving single stage have predominantly been employed in designing anaerobic system involving complex biomass. Recently, Linke [19] and Momoh and Nwaogazie [2] applied a first order biogas yield model in sizing continuous stirred tank and batch reactors respectively. In addition, the development of simplified kinetic models for more specific waste such as, organic fraction of municipal solid waste (OFMSW) has been reported , however, these simplified models were based on maximum specific bacteria growth rate ($\mu_{\max}$) [20,21].

In this study, a set of simplified kinetic models has been formulated by applying the biogas yield approach. This approach allows the estimation of various parameters such as recalcitrant fraction, biodegradable fraction, biodegradability and maximum biogas production rate. The model predictions have been assessed against the experimental biogas yield obtained by using representative samples of complex biomass.

2. Kinetic model development

The process of studying bacterial growth kinetics has been largely followed using the classical Monod growth kinetic model [22]. Though, this model has been established to be more appropriate in describing the growth process for pure culture utilizing homogenous substrates than for heterogeneous culture utilizing heterogeneous substrate [11,23], significant amount of studies on the kinetics of microbial growth and biodegradation involving mixed culture and complex substrates are still been described using the Monod growth kinetic model [22].

The heterogeneous nature of bacterial and the complex nature of substrate utilized in this study necessitated the consideration of other bacteria growth models such as the Moser's growth model [22] and its homologue, the Hill's growth kinetic model as proposed by Liu [22] and other inhibition models.

The model development involved the aggregation of hydrolysis/acidogenesis and acetogenesis/methanogenesis processes, and the process of biogas production was assumed to comprise (i) hydrolysis/acidogenesis by acidogenic bacteria to produce acidified substrate for the acetogenic/methanogenic ; (ii) uptake of acidified substrate by acetogenic/methanogenic bacteria and (iii) acidified substrate assimilation, growth and biogas production by the acetogenic /methanogenic bacteria. In this modeling approach, the substrate utilization model of Grau's [23] was used to describe the kinetics of the first two steps, while the process of substrate assimilation and growth of the acetogenic/methanogenic bacteria was studied by testing the Monod, Moser, Hill and Haldane's growth models.

2.1 Modeling hydrolysis/acidogenesis and growth of the acidogenic bacteria

The process of modeling the hydrolysis step using the first order kinetic model as reported by Eastman and Ferguson [24] has been described to be unsuitable for studying the digestion of complex biomass of co-digested substrate or more complex biomass [25,26,27]. In order to provide an appropriate description of the kinetics of hydrolysis, several researchers have modified the first order kinetic model as developed by Eastman and Ferguson [24].

Sanders et al. [28] developed a surface based kinetic model to describe the disintegration of complex substrate, however, this model failed to account for the recalcitrant fractions because it assumed the entire substrate to be biodegradable. Modification of the Sander's disintegration model was conducted by Esposito et al. [26] to describe the disintegration of organic fraction of municipal solid waste co-digested with sewage sludge. However, extensive characterization of complex biomass in terms of various characteristics, such as particle size distribution, carbonhydrates, proteins, lipids and inert was required for modeling the anaerobic process.

In this study, a simple substrate characterization model development was conducted that could provide an estimate of the recalcitrant fraction of complex biomass. Hydrolysis and acidogenesis were lumped together and they were assumed to be catalyzed by acidogenic bacteria releasing extracellular enzymes that are adsorbed on the surface of the complex biomass. The model of Grau [22, 23, 29] represented by Eq. (1a) which was subsequently modified as represented by Eq. (2) was adopted for modeling hydrolysis/acidogenesis.

$$\left| \frac{dS}{dt} \right| = \left| \frac{I_{\max(a)} X_{(a)}}{Y_{x/s(a)}} \right| \left| \frac{S}{S_o} \right|^n \quad (1a)$$

Where $-\frac{dS}{dt}$ represents the rate of change of complex substrate, and S represent the concentration of complex biomass volatile solids concentration remaining in effluent. S_0 represents the initial complex biomass volatile solid concentration, $\mu_{\max(a)}$ represents the maximum growth rate of acidogenic bacteria, X_a represents the acidogenic bacteria concentration, while $Y_{x/s(a)}$ represents the acidogenic bacteria biomass yield coefficient. However, in this study ($X_{(a)}$) was assumed to be constant such that Eq. (1a) could be re-written as

$$-\frac{dS}{dt} = K_{H(a)} \left[\frac{S}{S_0} \right]^n \quad \text{Eq. (1b)}$$

Where $K_{H(a)}$ is the maximum substrate utilization rate (g VS_{utilized}/L/day) for the acidogenic bacteria and “n” is the coefficient or degree of acidogenic bacteria adaptation for complex substrate degradation. Vavilin et al. [25] emphasized the importance of considering the recalcitrant fraction of complex biomass (R_f) when modeling hydrolysis of complex biomass thus, upon considering the recalcitrant fraction of complex biomass, Eq. (2) can be expressed as follows

$$-\frac{dS}{dt} = K_{H(a)} \left[\frac{S + S_0 R_f}{S_0} \right]^n \quad (2)$$

The term $K_{H(a)}$ is a measure of the maximum rate of volatile solids utilization by the acidogenic bacteria to produce acidified substrate while, the term “n”, could be viewed as a measure of the degree of volatile solids degradation by the acidogenic bacteria which, is largely dependent on the degree of bacteria adaptation. When “n” equals unity, the bacteria enzyme concentration is assumed to be in excess and the need for bacteria to adapt is not a pre-requisite for degradation. However, when “n” is greater than unity, enzyme concentration is assumed to be low such that, adaptation of the hydrolytic/acidogenic bacteria is a necessary pre-requisite for complex biomass degradation. The value

of “n” less than unity implies a poorly adapted acidogenic bacteria population and as the value of “n” approaches zero the reaction rate becomes independent of substrate concentration.

However, Eq. (2) can be expressed in terms of acidified substrate produced from the complex biomass as follows

$$\frac{dS_h}{dt} = K_{H(a)} \left(\frac{S + S_o R_f}{S_o} \right)^n \left(\frac{S_h}{S_o} \right) \quad (3)$$

Where S_h represents the concentration of acidified substrate solubilized from the complex biomass. It is worthy to note, that Eq. (3) is a modified form of the hydrolytic step as utilized by Barthakur et al. [11]; Faisal and Unno [30]; and Zinatizadeh et al. [31].

2.2 Uptake of acidified substrate by acetogenic/methanogenic bacteria

The uptake or utilization of the acidified substrate into acetogenic/methanogenic biomass was modeled using the first order Grau's substrate utilization model. In this modeling approach, uptake or utilization rate was considered to be inversely proportional to the initial volatile solids concentration; and directly proportional to the concentration of the active acetogenic/methanogenic biomass which was assumed to be constant ($X_{(a/m)}$, g/L)[23]; and also directly proportional to the difference in concentration of the acidified substrate generated by the acidogenic bacteria (S_h) and that remaining inside the cell of the acetogenic/methanogenic bacteria biomass ($S_{h(i)}$), such that, the substrate utilization rate of the acidified substrate by the acetogenic/methanogenic bacteria biomass can be represented by Eq. (4b – 4e).

$$\frac{dS_h}{dt} = \frac{dS_{h(u)}}{dt} + \frac{dS_{h(i)}}{dt} \quad (4a)$$

Re-arranging Eq. (4a)

$$\frac{dS_{h(u)}}{dt} = \frac{dS_h}{dt} - \frac{dS_{h(i)}}{dt} \quad (4b)$$

Expressing Eq. (4b) in terms of a first order Grau's model one obtains

$$\frac{dS_{h(u)}}{dt} = \left[\frac{K_{H(a/m)} X_{(a/m)}}{Y_{x/s(a/m)} S_o} \right] (S_h - S_{h(i)}) \quad (4c)$$

This can be re-written as Eq. (4d) at constant acetogenic/methanogenic bacteria concentration ($X_{a/m}$)

$$\frac{dS_{h(u)}}{dt} = \frac{K_{H(a/m)}}{S_o} (S_h - S_{h(i)}) \quad (4d)$$

Where $K_{H(a/m)}$ (g/L/day) is the maximum substrate utilization rate by the acetogenic/methanogenic bacteria while $(S_h - S_{h(i)})$ represents the concentration of acidified substrate taken up by the acetogenic/methanogenic bacterial biomass.

Expressing $S_{h(i)}$ in terms of S_h , Eq. (4d) can be re-written as

$$\frac{dS_{h(u)}}{dt} = \frac{K_{H(a/m)}}{S_o} (S_h - \alpha S_h) \quad (4e)$$

Where “ α ” is the fraction of S_h remaining intracellularly inside the acetogenic/methanogenic biomass if the hydrolyzed acidified substrate is not metabolized very fast, due to presence of inhibitory substances leading to accumulation of organic acids [32].

However, because the hydrolyzable/acidified substrate produced in Eq. (3) serves as substrate for the acetogenic/methanogenic bacteria biomass that was utilized fast enough, the intracellular concentration of the acidified substrate was assumed to be negligible ($S_{h(i)} = 0$),

Such that Eq. (4a) can be re-written as,

$$K_{H(a)} \left[\frac{S + S_o R_f}{S_o} \right]^n + \frac{S_h}{S_o} + \frac{K_{H(a/m)}}{S_o} S_h \quad (5)$$

Hence,

$$S_h + \left[\frac{S_o K_{H(a)}}{K_{H(a/m)} + K_{H(a)}} \right] \left[\frac{S + R_f S_o}{S_o} \right]^n \quad (6a)$$

Expressing S as a function of the initial influent volatile solids concentration Eq. (6a) can be re-written as

$$S_h + \left[\frac{S_o K_{H(a)}}{K_{H(a/m)} + K_{H(a)}} \right] \left[b + R_f \right]^n \quad (6b)$$

Where, b is the fraction of the initial substrate volatile solids concentration remaining in the effluent, (that is, $S = bS_o$)

$$\text{Assuming } \frac{K_{H(a)}}{K_{H(a/m)} + K_{H(a)}} = A_f \quad (7)$$

Where A_f represents the rate limiting step coefficient or solubilization fractional efficiency for the anaerobic process in which, the acidified substrate are metabolized very fast by the acetogenic/methanogenic bacteria such that, uptake by the acetogenic/methanogenic bacteria is not considered the rate limiting step. The rate limiting coefficient or solubilization fractional efficiency (A_f) can be viewed as a ratio between the maximum substrate utilization rate for production of acidified substrate by the acidogenic bacteria to the sum of the maximum substrate utilization rate for both the acidogenic and acetogenic/methanogenic bacteria population, such that, (A_f) may be expected to range from 0 -1.

Thus, Eq. (6b) may be re-written as

$$S_h = (S - R_f S_o) \left(\frac{K_{H(a)}}{K_{H(a)} + S_h} \right)^n \quad (8)$$

The term $(S - R_f S_o)$ represents the biodegradable substrate present in the volatile solids, however not all of these fractions are hydrolysable for uptake by the acetogenic/methanogenic bacteria cells due to environmental factors. Hence, (S_h) represent the actual amount of the substrate that was acidified and utilized by the acetogenic/methanogenic bacteria.

However, conditions may exist where the substrate utilization rate by the acetogenic/methanogenic bacteria become very slow such that the maximum substrate utilization rate by the acidogenic bacteria for production of acidified substrate becomes higher than the maximum substrate utilization rate for utilization of the acidified substrate by the acetogenic/methanogenic bacteria. For example, the acidic nature of the acidified substrate produce in excess can lead to decrease in pH, because high production of acidified intermediates can dissociate to produce protons which can compromise the neutral pH conditions required by the methanogenic bacteria for optimum performance [33], such that, $S_{h(i)} \neq 0$.

Thus, Eq. (8) can be expressed as Eq. (9) for very slow utilization of hydrolyzed acidified substrate by the acetogenic/methanogenic bacteria.

$$S_h = \left(\frac{S_o K_{H(a)}}{K_{H(a/m)} + S_h} \right) \left(\frac{K_{H(a)}}{K_{H(a)} + S_h} \right)^n \quad (9)$$

Hence, the rate limiting coefficient for very slow substrate utilization of acidified substrate by the acetogenic/methanogenic bacteria can be written as

$$\left(\frac{K_{H(a)}}{K_{H(a/m)} + S_h} \right) \left(\frac{K_{H(a)}}{K_{H(a)} + S_h} \right)^n = A_s \quad (10)$$

Where A_s is the rate limiting coefficient for very slow substrate utilization by the acetogenic/methanogenic bacteria elicited by presence of inhibitors which could be the acidified substrate in excess or other substances present in the acidified substrate.

It is worthy of note that, when the maximum substrate utilization rate for the acidogenic bacteria ($K_{H(a)}$) is less than that of the maximum substrate utilization rate for the acetogenic/methanogenic bacteria ($K_{H(a/m)}$), the rate limiting coefficient becomes less than 0.5 thus, implying hydrolysis/acidification as the rate limiting step. However, if the maximum substrate utilization rate for the acidogenic bacteria ($K_{H(a)}$) is greater than that of the maximum substrate utilization rate for the acetogenic/methanogenic bacteria ($K_{H(a/m)}$), the rate limiting coefficient becomes greater than 0.5, such that, acetogenesis/methanogenesis is considered the rate limiting step. In addition, if the maximum substrate utilization rate for the acidogenic bacteria ($K_{H(a)}$) is equal to that of the maximum substrate utilization rate for the acetogenic/methanogenic bacteria ($K_{H(a/m)}$), the rate limiting coefficient becomes equal to 0.5.

2.3 Assimilation and growth of acetogenic/methanogenic bacteria

In modeling the assimilation and growth process of the acetogenic/methanogenic step, the growth models of Monod, Moser, Hill, and Haldane were considered. Although, the growth model of Monod has predominantly been used to describe growth processes for low substrate concentration, the possibility of a Moser's and more recently, the Hill's growth model as proposed by Liu [22] to describe growth kinetic at low substrate concentration had to be considered because of the complex nature of the substrate and mixed culture of micro-organism. For growth processes affected by acidity of the acidified substrate, the growth model of Haldane (Andrews) was employed [34]. Also for growth process affected by the allosteric effectors present in the acidified substrate, the Haldane (Non-competitive) model as described by Noykova et al. [35] was utilized to describe the growth process.

The acetogenic/methanogenic bacteria have been reported to have a minimum doubling time of about 1-4 days [36], however, for the sake of simplicity, these bacteria were lumped together. The process following assimilation of acidified substrate led to cell growth of acetogenic/methanogenic bacteria and production of biogas (methane and carbondioxide). Similar process of lumping acetogenic/methanogenic bacteria was reported by Vavilin et al. [37] and Vavilin and Angelidaki [38] for modeling anaerobic digestion of solid waste.

The yield coefficient for biogas yield has been represented as

$$Y_{y/s} = \frac{dy_t}{dS} \quad (11)$$

And, the yield coefficient for biomass production was represented as

$$Y_{x/s(a/m)} = \frac{dX_{(a/m)}}{dS} \quad (12)$$

The Monod growth model for the acetogenic/methanogenic bacteria can be represented by

$$\mu = \frac{\mu_{\max(a/m)} S_h}{K_s + S_h} \quad (13)$$

But acetogenic/methanogenic bacteria growth rate can be represented as

$$\frac{dX}{dt} = \mu X_{(a/m)} \quad (14)$$

However, the yield coefficient for acetogenic/methanogenic bacteria growth can be expressed as

$$\frac{\left| \frac{dX}{dt} \right|}{\left| \frac{dS}{dt} \right|} = Y_{x/s(a/m)} \quad (15)$$

Thus, substrate utilization rate can be represented as

$$\frac{dS}{dt} = \frac{1}{Y_{x/s(a/m)}} \frac{dX}{dt} \quad (16)$$

Hence, Eq. (16) can be re-written as

$$\frac{dS}{dt} = \frac{1}{Y_{x/s(a/m)}} \frac{X_{(a/m)} \mu_{\max(a/m)} S_h}{k_s + S_h} \quad (17)$$

Similarly, the yield coefficient for biogas yield by the acetogenic/methanogenic bacteria can be represented as

$$\frac{\frac{dy_t}{dt}}{\frac{dS}{dt}} = Y_{y/s} \quad (18)$$

Such that, the biogas yield rate can be represented as

$$\frac{dy_t}{dt} = Y_{y/s} \frac{dS}{dt} \quad (19)$$

Substituting Eq. (17) into Eq. (19) one obtains

$$\frac{dy_t}{dt} = \frac{Y_{y/s}}{Y_{x/s(a/m)}} \frac{X_{(a/m)} \mu_{\max(a/m)} S_h}{k_s + S_h} \quad (20)$$

It is important to note that the growth rate of the acetogenic/methanogenic bacteria was assumed to be

very slow or relatively constant such that $\frac{\mu_{\max(a/m)} X_{(a/m)}}{Y_{x/s(a/m)}}$ was replaced with the term $K_{H(a/m)}$, (g VS

utilized/L/day) which represent the maximum substrate utilization rate by the acetogenic/methanogenic

bacteria. Additionally, the death rate of the acetogenic/methanogenic bacteria (k_d , /day) was assumed

to be negligible due to the slow growth rate of these micro-organisms. Furthermore, the multiplication

of $Y_{y/s}$ ((mL_{biogas}/g VS)/(g VS_{utilized}/L)) and $K_{H(a/m)}$ resulted in the maximum specific biogas yield rate

$(R_{\max})$ (mL_{biogas}/g VS/ day), while $\frac{dy_t}{dt}$ (mL_{biogas}/g VS/ day) can be described as the specific biogas yield rate (R) at the end of biogas production.

Thus, Eq. (20) can be re-written as

$$R = \frac{Y_{y/s} K_{H(a/m)} S_h}{k_s + S_h} \quad (21)$$

Hence, by substituting Eq. (8) into Eq. (21) one obtains

$$R = \frac{R_{\max} A_f S_o (b + R)^n}{k_s + A_f S_o (b + R)^n} \quad (22)$$

Eq. (22) can be used to describe the biogas yield rate from complex biomass considering acidified substrate as limiting. However, Eq. (22) can be re-arranged so that the volatile solids apparently appear to be the limiting substrate as represented by Eq. (23)

$$R = \frac{R_{\max} S_o}{\frac{k_s}{A_f (b + R)^n} + S_o} \quad (23)$$

The term k_s represents the Monod half saturation constant for the acidified substrate while $\frac{k_s}{A_f (b + R)^n}$ represents the Monod half saturation constant in volatile solids equivalent which can be represented as K_s .

Similar process was applied to develop the Moser's based biogas yield rate model by assuming that the growth process of the acetogenic/methanogenic bacteria can be described using the Moser's growth model represented by Eq. (24).

$$R = \frac{R_{\max} S_h^m}{k_s + S_h^m} \quad (24)$$

Thus, the Moser's based biogas yield rate model becomes

$$R = \frac{R_{\max} S_o^m}{\frac{k_s}{A_f^{1/m} b + R_f^{1/mn}} + S_o^m} \quad (25)$$

Where, “m” represents the degree of acetogenic/methanogenic bacterial adaptation for cooperativity, which should always be greater than unity ($m > 1$) as described by Moser [22]. Again, the Moser's growth kinetic model can be re-arranged to appear as a Hill's function as proposed by Liu [22] represented by Eq. (26),

$$R = \frac{R_{\max(a/m)} S_h^m}{k_n^m + S_h^m} \quad (26)$$

The Hill's based biogas yield rate model was developed as represented by Eq. (27) by following similar derivation as conducted for the Monod based biogas yield rate

$$\text{model. } R = \frac{R_{\max} S_o^m}{\frac{k_n^m}{A_f^{1/m} b + R_f^{1/mn}} + S_o^m} \quad (27)$$

It is important to note that k_n represents the Hill's half saturation constant and $\frac{k_n^m}{A_f^{1/m} b + R_f^{1/mn}}$ represents the Hill's half saturation constant in volatile solids equivalent which can be represented as K_n .

In cases where the acidic nature affects the utilization of acidified substrate, the Haldane's (Andrews) growth model [34, 39] represented by Eq. (27) was employed to describe bacteria growth.

$$R = \frac{I_{\max(a/m)} S_h}{S_h + k_s + \frac{S_h^2}{k_i}} \quad (27)$$

In this growth process, the acidic nature of acidified substrate may affect its metabolism such that substrate utilization is slow but not necessarily the rate limiting step (A_f) or very slow to become the rate limiting step (A_s). Thus, the Haldane's (Andrews) based biogas yield rate model can be represented by the Eq. (28).

$$R = \frac{R_{\max} S_0}{S_0 + \frac{k_s}{A_f + b + R_f^n} + \frac{S_0^2 A_f + b + R_f^n}{k_i}} \quad (28)$$

The Haldane (non-competitive) growth rate model assumes that the acidified substrate may non-competitively affect growth process through allosteric mechanisms. Haldane (non-competitive) growth rate model can be described by Eq. (29) [35, 40].

$$R = \frac{I_{\max(a/m)} S_h}{S_h + k_s + \left(1 + \frac{S_h}{k_i}\right)} \quad (29)$$

In this form, the allosteric nature of the acidified substrate may affect its metabolism such that substrate utilization is slow but not necessarily the rate limiting (A_f) or very slow to become the rate limiting step (A_s). Here, the affinity for the acidified substrate is not affected but its utilization is hindered [41]. Thus, the non-competitive Haldane based biogas yield rate model can be represented as

$$R = \frac{R_{\max} S_0 A_f + b + R_f^n}{k_s + S_0 A_f + b + R_f^n + \frac{S_0 A_f + b + R_f^n}{k_i}} \quad (30)$$

The various model parameters were evaluated using the solver function of the Microsoft Excel tool Pak and the most appropriate models were selected based as their high correlation coefficient and low root

mean square error (RMSE). In situations where more than one model share similar correlation coefficient and RMSE, the second-order Akaike's information criterion (AIC_c) was employed to compared these models [39, 42].

$$AIC_c = 2K + n^* \log \left| \frac{SS_{reg}}{n^*} \right| + \frac{2K(K+1)}{n^*(K+1)} \quad (31)$$

Where SS_{reg} is the residual sum of square represented by $\sum (d_i - f(x))^2$ and d_i is the experimental data while $f(x)$ is the estimated data of the fitted model [39]. The number of available points was represented by n^* , while, K represented the number of parameter to be estimated. When the difference in AIC_c between two models is less than 2, no difference is believed to exist between the models thus, both models could be used to represent the given data points [39].

3. Materials and methods

3.1 Substrate collection

The raw material utilized in this study comprised cow manure and waste paper. Cow manure was obtained from abattoir situated at Choba Community, Rivers State (Nigeria) and waste paper was obtained from dumpsites situated at the University of Port Harcourt, Rivers State Nigeria. About 500g of cow manure was collected and sun dried at ambient temperature for a period of 20 days; it was subsequently crushed using a mortar and pestle and about 500g of waste paper was sun dried which was afterwards ground to fine particles using a grinding mill. The volatile solids content and carbon to nitrogen ratio were determined according to APHA [43]. Volatile solids for cow manure and waste paper were determined to be 66.1% and 85.7% respectively using a muffle furnace, Carbolite model LMF 4 manufactured in England, and carbon to nitrogen ratio was determined to be 22:1 and 150:1 respectively.

3.2 Experimental methodology

In this approach of studying the anaerobic biodegradability of complex biomass, the experimental work was conducted in two phases. The first phase was designed to optimize the substrate mix proportion of cow manure and waste paper and the second phase was designed to maximize biogas production from the optimal mix proportion obtained in the first phase of the experimental work.

3.2.1 Experimental procedure for substrate optimization

The experiment was conducted using five Buchner flasks operated in a batch mode. A split plot design approach as utilized by Shin et al.[44] comprising a total of 5 treatments of cow manure and waste paper were mixed in the ratio of 100:0 (A1), 75:25 (A2), 50:50 (A3), 25:75 (A4) and 0:100 (A5). The substrates were loaded in the Buchner flasks each with volumetric capacity of 500mL containing 250mL of water and corked to exclude air. The experiments were conducted in duplicates and were allowed to run at an average ambient temperature of $30\pm 3^{\circ}\text{C}$ and the pH of the digesters are as shown in Table 1. The biogas produced was measured by water (brine solution) displacement method and agitation of the batch reactors was carried out twice daily. The biogas produced was analyzed using Gas Chromatography Agilent Technologies Model 1890A. The total solids content loaded in all digesters was fixed at 6.5% which was within the recommended range of 4 – 12% for low solid loading anaerobic digestion [45].

3.2.2 Experimental procedure for biogas maximization

In order to maximize biogas production from the optimized substrate mix obtained in the experiment described above, nine sets of batch digesters comprising cow manure and waste paper mixture in proportion of 75:25 were set up in batch digesters labeled B1, B2, B3, B4, B5, B6, B7, B8 and B9, which consisted of total solids concentration 1, 2, 3, 4, 5, 6, 7, 8 and 9% respectively. The digesters

were setup as described by Momoh and Nwaogazie [3] and were also conducted in duplicates and allowed to run at average ambient temperature of $28 \pm 4^{\circ}\text{C}$.

4. Results and Discussion

In the first phase, a retention time of about 40 days was maintained in almost all the digesters studied, and the mixture of cow manure and waste paper combined in the proportion of 75:25 (A2) produced the highest quantity of biogas ($921 \pm 12\text{ mL}$) and the methane content was determined to be $58 \pm 3\%$ or $534.15 \pm 12\text{ mL}$ of methane (Table 1). The batch digester comprising of cow manure alone (A1) produced $421 \pm 10\text{ mL}$ of biogas with methane content of $52 \pm 2\%$ or $218.92 \pm 10\text{ mL}$ of methane. The digesters A3, A4, and A5 had insignificant quantity of methane in the biogas produced. The low methane content in these digesters could be attributed to shock or instability due to high volatile acid formation following the hydrolysis of waste paper. The high performance of digester A2 strongly underscores the benefits of co-digestion in this study which may include reduced toxicity, nutrient balance and microbial synergism [8].

Table 1: Digester Characteristics and Biogas Composition

In the second phase of the experiment, the process of maximizing biogas yield from this optimal mix of cow manure and waste paper (75:25) determined in this study was conducted in nine (9) digesters that comprised total solids ranging from 1 – 9%. After 80 days retention time, the biogas yield and specific biogas yield rate were observed to increase as substrate concentration increased from 1-4%, but remained almost steady for substrate concentration from 5 -9% (Fig. 1). However, digester B3 exhibited difficulty in producing significant amount of biogas and hence it was eliminated from the

study. The longer retention time experienced in the second phase may be attributed to a reduced average ambient temperature of $28 \pm 4^\circ \text{C}$.

Fig.1 – Biogas yield and specific biogas yield against total solids concentration.

4.1 Kinetics and biodegradability parameter estimation and model validation

The process of characterizing the optimal mix proportion of cow manure and waste paper (75:25) involved the application the biogas yield rate models of Monod, Moser, Hill and Haldane's models as illustrated in this study. The kinetic and biodegradability parameters estimated in this study include;

- (a) Monod half saturation constant for the acidified substrate (k_s) (g/L).
- (b) Monod half saturation constant in volatile solids equivalent (K_s) (g/L).
- (c) Hill's half saturation constant for the acidified substrate (k_n) (g/L).
- (d) Hill's half saturation constant in volatile solids equivalents (K_n) (g/L).
- (e) Maximum specific biogas yield rate ($R_{\max}$) (mL/g VS/day)
- (f) The coefficient "m"
- (g) The coefficient "n"
- (h) Fraction of volatile solid remaining in effluent (b)
- (i) The recalcitrant fraction (R_f).
- (j) Fraction of biodegradable volatile solids ($1-R_f$)
- (k) Fraction of biodegradable volatile solids remaining in effluent ($b-R_f$)
- (l) Biodegradability ($1-b$)
- (m) Rate limiting coefficient for fast or very slow uptake of acidified substrate ($A_{f(s)}$)

The results of parameter estimation using non-linear regression are presented in Table (2). It was observed that the five models tested in this study can be utilized to characterize anaerobic biodegradation kinetics because each provided a high correlation coefficient (r) of 0.99. However, the process of selecting the most appropriate model resided in the observance of the root mean square error (RMSE). Models with the lowest root mean square error (RMSE) are normally considered more appropriate to describe a given data set if they share similar correlation coefficient.

The five models tested in this study produced correlation coefficient (r) of 0.99 each, however, the Moser and Hill's based biogas yield rate models provided the lowest root mean square error (RMSE) of 5.87×10^{-3} each, while the Monod, Haldane (Andrews), Haldane (non-competitive) based biogas yield rate models provided higher RMSE of 0.0428, 0.0256 and 0.0256 respectively. Thus, only the Moser and Hill's based biogas yield rate models were considered most appropriate in describing the specific biogas yield rate from this biomass mixture because they provided the least RMSE.

However, because these selected models produced similar correlation coefficient (r) and RMSE, a second-order Akaike's information criterion (AIC_c) [42] was employed to assess model superiority. Upon computation, the second-order Akaike's information criterion analysis produced again, similar AIC_c value of 97.33 each, for both models (Table 3) implying that, both models have the potential to be utilized in studying the anaerobic biodegradation kinetics of this biomass mixture. Hence, subsequent discussions were limited to the biogas yield rate models of Moser and Hill's.

It is interesting to note that the Moser and Hill's growth rate models which formed the basis for these selected models could be described as homologues in which, the characteristic coefficient ' m ' (which is

always greater than unity) differentiates them from the Monod growth models where “ m ” is equal to unity. Moser considered this coefficient ‘ m ’ to be related more to adaptation of microbial population to environmental condition through process of mutation [40] while Liu [22] proposed that the coefficient may well be related to cooperativity among microbial species – substrate pairs. However, because bacteria grown under substrate limiting conditions may tend to adapt through process of mutation by modification at the phenotypic and genetic levels that may lead to improve transport for growth limiting substrate and/or improve cooperativity among adapted microbial species [23] the views held by these researchers may not be farfetched.

Table 2- Parameter estimate for developed biogas yield rate models

In essence, by choosing the Moser’s biogas yield rate model, the Monod half saturation constants for the acidified substrate (k_s) and the Monod half saturation constant in volatile solid equivalent (K_s) were estimated to be 0.1558 and 1.637g/L respectively. This estimated Monod half saturation constant for the acidified substrate (k_s) compares reasonably with values of 0.143-0.207g/L reported by Barthakur et al. [11] for half saturation constant for acetate by the methanogenic bacteria population. Also, the estimated k_s , lies within the range of 0.1- 0.41g/L reported by Pavlostathis and Giraldo-Gomez [41] as

half saturation constant displayed by acetoclastic methanogens. Furthermore, the biodegradability parameters estimated using this model revealed that the recalcitrant fraction in this biomass mixture was 0.267 of the initial volatile solids fed, and the biodegradable fraction ($1-R_f$) was 0.733 of the initial

volatile solids fed. The biodegradability ($1-b$) was 0.1925 while the biodegradable fraction remaining ($b-R_f$) was 0.540 of the initial volatile solids fed at ambient temperature conditions. It is interesting to note that the sum of the biodegradable fraction remaining at the end of experiment ($b-R_f$) and biodegradability potential ($1-b$) must be equal to the biodegradable fraction of the feedstock volatile solids ($1-R_f$).

However, by choosing the Hill's based biogas yield rate model, the Hill's half saturation constant for the acidified substrate (k_n) was estimated to be 0.2288. Although, no study exist in literature that has applied the Hill's growth model in studying the kinetics of bacteria growth after it was proposed by Liu [22], the possibility of this type of kinetics cannot be overruled because the estimated Hill's half saturation constant (k_n) seems to bear some semblance with the Monod half saturation constant (k_s). In addition, the Hill's half saturation constant in volatile solid equivalents of 5.34gVS/L obtained in this study compares reasonably to the value of 5g VS/L reported by Angelidaki et al. [46] for household solid waste using the Monod growth model for the acetoclastic methanogens.

Moreover, the recalcitrant fraction (R_f) was estimated to be 0.371 which is close to 0.400 reported by Barthakur et al. [11] and Hashimoto [47] for cow manure alone. In addition, the biodegradable fraction ($1-R_f$) was calculated to be 0.628 of the initial volatile solids fed while the biodegradability ($1-b$) was calculated to be 0.3136, and the biodegradable fraction remaining in the effluent ($b-R_f$) was calculated to be 0.3154 of the initial volatile solids concentration.

Furthermore, both models seem to indicate certain degree of bacterial adaptation for substrate degradation and cooperativity among the acidogenic and acetogenic/ methanogenic bacterial population because “ n ” and “ m ” were greater than unity. The coefficients of adaptation for degradation by

acidogenic bacteria (n) considering the Moser and Hill's based biogas yield rate models were 1.732 and 1.360 respectively, while the coefficient of adaptation for cooperativity by the acetogenic/methanogenic bacterial (m) was estimated to be 2.738 each for both the Moser and Hill's based biogas yield rate models. Because these coefficients were higher than unity, some degree of bacterial adaptation and/or cooperativity was implied. Adaptation is a necessary biological process associated with micro-organisms when grown under substrate limiting conditions [23].

In this study, the importance of the terms "n and m" cannot be overemphasized. The term "m" may be defined as coefficient of adaptation for cooperativity by the acetogenic/methanogenic bacteria, while, the coefficient "n" can be described as the degree of adaptation for complex biomass degradation by the hydrolytic/acidogenic bacteria. Thus, consideration of bacteria adaptation for cooperativity "m" amongst the acetogenic/methanogenic species and bacteria adaptation for complex substrate degradation "n" amongst the hydrolytic/acidogenic bacteria species may contribute significantly in the entire process of modeling biogas yield production rate from complex biomass.

In addition to the AIC_c for model selection, further improvement in model selection was conducted by comparing the percent error between the graphically observed half saturation constant and the half saturation constants in volatile solids equivalents estimated through the modeling approach. The half saturation constant in volatile solids equivalent is described as the substrate volatile solids concentration corresponding to $0.5R_{max}$. The corresponding saturation constants in volatile solids equivalent are shown in Table 3 while, the combined curve fitting for the tested models is shown in Fig. 2.

Fig.2- Combined graphs of specific biogas yield rate against volatile solids concentration

Table 3- Model selection technique showing AIC_c and Percent error for selected Models

Upon comparison of the percent errors, it was observed that the Hill's based biogas yield rate model provided a lower percent error of 2.91% when compared to the 72.71% obtained from the Moser's based biogas yield rate model (Table 3). Thus, the Hill's based biogas yield rate model may provide a reasonable description of the half saturation constant in volatile solids equivalent (K_n) better than the Moser's based biogas yield rate model.

Moreover, the utilization of the linear plot similar to the so called "Lineweaver-Burks" encountered in enzymology revealed that, the plot of the inverse of the specific biogas yield rate obtained at the end of the experiment ($1/R_e$) against the inverse of the initial substrate volatile solids concentration ($1/S_0$) yielded a linear curve fitting (Fig.3) with slope equal to (K_n/R_{max}) and intercept equal to ($1/R_{max}$). The solutions for the maximum specific biogas yield rate (R_{max}) and half saturation constant (K_n) were 2.3ml/ g VS/day and 5.2g VS/L respectively, which compare reasonably to that estimated by the Hill's based biogas yield rate model than for the Moser's biogas yield rate model.

Fig. 3- Plot of the inverse of the specific biogas yield rate ($1/R_e$) against the inverse of the substrate volatile solids concentration ($1/S_0$)

In essence, the Hill's based biogas yield rate model may be viewed as most appropriate in studying biogas production from this biomass mixture. By utilizing this model, the maximum specific biogas yield rate estimated as 2.2 mL/g VS/day seem to compare reasonably with the value of 1.75mL/g VS/day obtained by Budiyono et al. [48] from the digestion of cow manure alone at ambient temperature while, the substrate concentration corresponding to this maximum biogas yield was

observed at 70g VS/L. The high rate of biogas production from this mixture may be attributed to the benefits associated with co-digestion which include the provision of effective buffering system, nutrient balance, microbial synergism and reduction in toxicity linked with anaerobic digestion [8]. In addition, the ability for the bacteria to adapt and cooperate in utilization of substrate may have strongly influenced the rate of biogas production as highlighted in this study.

In general, it is important to note that the Contois and two phase kinetic models which can also be used to model hydrolysis [24] were inapplicable in this study because these models are directly dependent on bacteria biomass concentration which was not feasible to evaluate in the study due to the difficulty involved in differentiating between bacteria biomass and complex biomass volatile solids. The utilization of an n^{th} -order model of Grau [22, 23, 29] as applied in this study enabled for the integration of bacteria behavior into the n^{th} power. Also, assimilation of acidified substrate and growth of acetogenic/methanogenic bacteria was observed to be most appropriately described by the Hill's growth model as against the Monod's growth model that was originally developed for pure culture utilizing homogenous substrate [23]. Thus, there may be need to consider the Hill's growth model during anaerobic degradation of complex biomass especially for co-digested complex substrates especially where improved biogas yield has been reported [8].

4.2 Application of kinetic models

4.2.1 Appropriate replacement for first order models

The approach to modeling anaerobic digestion has been grouped into three broad categories by Tomei et al. [17]. This includes simple substrate characterization models; intermediate substrate characterization models and advance substrate characterization models. The simple substrate characterization models do not distinguish between different components of the substrate into protein

carbohydrates, lipid etc., and they are the rate limiting type models. However, the advance substrate characterization models require the substrate be characterized into carbohydrates, proteins, lipids, etc. before they can be utilized. In addition, the input parameters needed to implement these type of models are usually numerous. Example of advanced type models include the models developed by Angelidaki et al. [16] and Anaerobic Digestion Model No. 1 [2].

In this study, it is evident that a simple substrate characterization model has been developed that describes biogas production from complex biomass. This modeling approach has the advantage of providing sufficient information about the anaerobic digestion kinetics and the nature of substrate undergoing anaerobic decomposition from very little input data. In addition, this approach eliminates the need to quantify the viable bacteria biomass volatile suspended solids which is usually very difficult to estimate for complex biomass [9] and a necessary requirement when utilizing the Contois and the two phase base models [22].

Traditionally, the first order models which are examples of simple substrate characterization models have largely been employed in the well known “biochemical methane potential” assay (BMP) and also in the design of anaerobic systems to evaluate anaerobic biodegradability and plant design. Though, first order models are easy to handle, they fail to provide any information about substrate concentration required for maximum biogas production. However, with the modeling approach developed in this study, the biochemical methane potential assay can be evaluated in a more holistic manner. In addition, the substrate concentration corresponding to maximum biogas yield can easily be estimated thus, contributing to the design and optimization of anaerobic process.

4.2.2 Determination of carbon flux and the rate limiting coefficient ($A_{f(s)}$)

In a multi-step process, the step which limits or controls the rate of the overall process is called the rate limiting step [41]. In anaerobic digestion with its multi- step processes, the hydrolysis is usually assumed to be the rate limiting step in the anaerobic digestion of particulate or complex biomass [24], and the methanogenesis step is considered to be the rate limiting step for the anaerobic digestion of soluble substrates [40].

In this study, it was possible to utilize numeric values to approximate the rate limiting step such that the identification of hydrolysis/acidogenesis step was less tedious as value of (A_f) less than 0.5 confirmed hydrolysis/acidogenesis as rate limiting step in the anaerobic digestion of the biomass mixture.

The Fig. 4 shows the effect of carbon flux on biogas yield rate, in which the fractional proportion of the maximum biogas yield rate (R/R_{max}) was plotted against the initial volatile solids concentration utilized in this study (S_0).

Fig.4-Fractional proportion of the maximum biogas yield rate against the volatile solids concentration for the Hill's based biogas yield rate model.

It was observed that the volatile solids concentration within 30 – 70g VS/L may be appropriate for maximizing biogas production while concentration below this range was observed to reduce biogas production from this biomass mixture at ambient temperature. Furthermore, it can be observe that conditions which tend to restrict hydrolysis that is, reduce the value of the rate limiting coefficient or solubilization fractional efficiency (A_f) (such as, during the digestion of recalcitrant substrate) can reduce fractional biogas production rates at low volatile solid concentration below 30g VS/L. On the

other hand, conditions that tend to improve hydrolysis that is, increase the value of the rate limiting coefficient (A_f), (such as, during the digestion easy hydrolysable substrates or physiochemical pre-treatment of complex biomass) can lead to an increased fractional biogas production rates at low volatile solids concentration below 30g VS/L. These findings clearly explain why mechanical treatment of complex biomass may tend to enhance biogas production [49, 50].

In general, the restriction or ease of carbon flow through the interlinked biochemical reactions may play a crucial role in the determination of the rate limiting step and also, biogas production rate during the anaerobic digestion of complex biomass. These findings tend to give credence to the works of Pavlostathis et al. [41] who reported that the rate limiting step may change depending on the nature of substrate and other factors.

5. Conclusions

In this study, the co-digestion of cow manure and waste paper (75:25) was observed to result in an increase in biogas production when compared to the digestion of these substrates alone. The process of studying the kinetics and biodegradability of this optimal mixture revealed that the Hill's based biogas yield rate model was most appropriate in describing the biogas production from this mixture of complex biomass. The developed Hill's based biogas yield rate model was able to account for adaptation by the acidogenic bacteria to degrade complex biomass (n) and also the adaptation for cooperativity by the acetogenic/methanogenic species (m) to assimilate acidified substrate. The half saturation constants obtained using this model showed comparable values to that obtained when acetate was considered growth limiting. The biodegradability, biodegradable fraction and recalcitrant fraction

were estimated to 0.3136, 0.628 and 0.371 respectively while, the rate limiting coefficient was estimated to be 0.205 implying that, hydrolysis was rate the limiting step.

In general, this modeling approach seems to breach the gap between the simplified first order models and the advance substrate characterization models, and it may provide more benefits in designing of anaerobic systems as compared to the first order modeling approach. Additionally, the application of the modeling approach in the biochemical methane potential assay may provide advanced information about the biodegradability of biomass utilized in anaerobic digestion.

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Fig.1 – Biogas yield and specific biogas yield against total solids concentration.

Fig.4-Fractional proportion of the maximum biogas yield rate against the volatile solids concentration for the Hill's based biogas yield rate model.

Fig.2- Combined graphs of specific biogas yield rate against volatile solids concentration

Fig. 3- Plot of the inverse of the specific biogas yield rate ($1/R_c$) against the inverse of the substrate volatile solids concentration ($1/S_0$)

Table 1: Digester Characteristics and Biogas Composition

Digester	Mix proportion	Weight of cow manure (g)	Weight of waste paper (g)	Conc. Volatile solids (g/L)	pH	Cumulative biogas (mL)	CH ₄ (%)	CO ₂ (%)
A1	100:0	17.40	0.00	46.00	7.3! 0.04	421! 10	52! 2	48±2
A2	75:25	13.05	4.35	49.36	7.3! 0.02	921! 12	58! 3	42±3
A3	50:50	8.70	8.70	52.80	7.2! 0.03	164! 22	10! 3	90±3
A4	25:75	4.35	13.05	56.20	7.2! 0.04	152! 23	9.0! 3	91±3
A5	0:100	0.00	17.4	59.64	7.1! 0.03	260! 34	12! 3	88±3

Table 2- Parameter estimate for developed biogas yield rate models

Biogas yield rate models	$R_{\max}$ mL/gVS/day	k_s g/L	k_n (g/L)	k_i (g/L)	$K_s \left(1 + \frac{k_s}{A_f b! R_f!} \right)$ (g/L)	$K_n \left(1 + \frac{k_n}{A_f b! R_f!} \right)$ (g/L)	m	n	R_f	b	$A_{f(s)}$	RMSE
Monod based	2.358	0.127	-	-	3.260	-	-	1.484	0.266	0.649	0.161	4.28E-02
Moser's based	2.200	0.155	-	-	1.637	-	2.738	1.732	0.267	0.807	0.2762	5.87E-03
Hill's based	2.200	-	0.228	-	-	5.34	2.738	1.360	0.371	0.6864	0.205	5.87E-03
Haldane (Andrew) based	2.732	0.268	-	20.230	5.123	-	-	1.158	0.326	0.787	0.128	2.56E-02
Non- competitive Haldane based	2.769	0.209	-	15.317	5.200	-	-	1.718	0.282	0.775	0.1351	2.56E-02

Table 3- Model selection technique showing AIC_c and Percent error for selected Models

Biogas yield rate model	Equations	Number of parameters	Observed (graphical) K _s or K _n (g/L)	Estimated K _s or K _n (g/L)	AIC _c	Percent error (%)
Monod's based	$R = \frac{R_{\max} S_o}{k_s + R_f^n + S_o}$	6	3.500	3.200	-	-
Moser's based	$R = \frac{R_{\max} S_o^m}{k_s + R_f^{mn} + S_o^m}$	7	6.000	1.637	97.33	72.71
Hill's based	$R = \frac{R_{\max} S_o^m}{k_s + R_f^{mn} + S_o^m}$	7	5.500	5.340	97.33	2.91
Haldane (Andrews) based	$R = \frac{R_{\max} S_0}{S_o \left(\frac{k_s}{A_f^{ls} + R_f^n} + \frac{S_0^2}{A_f^{ls} + R_f^n} \right) + k_i}$	7	4.000	5.123	-	-
Non-competitive (Haldane)	$R = \frac{R_{\max} S_o A_f^{ls} + R_f^n}{k_s + S_o A_f^{ls} + R_f^n + \frac{S_o A_f^{ls} + R_f^n}{k_i}}$	7	4.000	5.200	-	-

Highlights

- !! Co-digesting cow manure and waste paper (75:25) optimized biogas.**
- !! Hill's based biogas yield rate model described experimental data.**
- !! Half saturation constant was estimated as 0.228g/L.**
- !! Biodegradable and recalcitrant fractions were 0.628 and 0.371 respectively.**