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$$y_t = y_m - \frac{y_m (k'_o S_o + b)}{k'_o S_o + b \exp \left((k'_o S_o + b) \left(\frac{(t+1)^{1-h} - 1}{1-h} \right) \right)}$$

**DEVELOPMENT OF A NOVEL FRACTAL-LIKE KINETIC MODEL FOR ELUCIDATING THE
EFFECT OF PARTICLE SIZE ON THE MECHANISM OF HYDROLYSIS AND BIOGAS YIELD
FROM LIGNO-CELLULOSIC BIOMASS**

BY

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Nomenclature

$b = \left(\frac{X_o}{Y} k'_o \right)$ = initial hydrolytic rate constant (day^{-1})

d = overall catalytic rate (day^{-1})

k_o, k^*, k' = rate constant in ideal homogenous solution (L/g/day)

k'_o = specific affinity constant in ideal homogenous solution (L/g/day)

$k'_o S_o$ = overall affinity constant (day^{-1})

k_t = rate coefficient in topological constrained environment (day^{-1})

t = time (days)

h = fractal exponent

S_o = initial volatile solids concentration (g/L)

S = volatile solids concentration remaining (g/L)

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

Y = yield coefficient

y_m = maximum biogas yield (L/g VS)

y_t = biogas yield (L/g VS)

R_m = maximum specific biogas yield rate (L/g VS /day)

X = hydrolytic biomass concentration (g/L)

X_o = initial hydrolytic biomass concentration (g/L)

μ_m = Maximum specific bacteria growth rate (day^{-1})

μ_m/K_s = specific affinity (L/g/day)

λ = lag phase (days)

ABSTRACT

The effect of particle size on hydrolysis and biogas production kinetics from a typical ligno-cellulosic biomass was assessed by studying the anaerobic co-digestion of fixed amount of rice husk and cow dung mixture using a newly developed fractal-like kinetic model. British Standard (BS) sieves were used to obtain varying particles size fractions ranging from 0.150 - 0.212mm, 0.212 - 0.300mm, 0.300 - 0.600mm, 0.600 - 1.000mm and 1.000 - 1.700mm from oven dried, milled rice husk and pulverized, dried cow dung respectively. These particle size fractions from both biomass were mixed in a ratio of 1:1 after which, they were loaded into batch reactors and digested anaerobically at ambient conditions for 75 days. Hydrolysis of ligno-cellulosic biomass was observed to depend on the fractal exponent (h), which indexed the presence of inaccessible regions in ligno-cellulosic biomass. Also, hydrolysis was observed to depend on two other intrinsic factors that comprised of the initial hydrolytic rate $\left(\frac{X_o}{Y} k'_o\right)$ and overall affinity constant $(k'_o S_o)$. Larger particle size fractions were associated with higher affinity but lower initial hydrolysis rate while, smaller particle size fractions were associated with lower affinity but higher initial hydrolysis rate. In addition, the fractal model compared favorably with the popular modified Gompertz equation.

Keywords: Fractal-like kinetics, fractal exponent, particle size, biogas yield, inaccessible regions

1. Introduction

Anaerobic digestion has been described as an appropriate technology with the potential of converting organic waste of high nuisance value into environmental benefits (Vaa, 1993). The environmental benefits are not only restricted to improved environmental quality but also, the production of essential fuel, rich in methane (50-70%) (Igoni 2007, Angelidaki et al., 1999) that can serve as a viable source of low carbon foot print energy in the form of biogas. The utilization of complex ligno-cellulosic biomass as feedstock for anaerobic digestion has continued to attract attention possibly due to the fact that, ligno-cellulose accounts for approximately 50% of the biomass in the world and the production of lignocellulose can amount up to about 200 billion tons per year (Claassen et al., 1999; Zhang, 2008). Ligno-cellulosic biomasses can be described as plant product, which consist of large macro-molecules with irregular, porous and rough surfaces (Congcong et al, 2015), comprising mainly of three biopolymers that include; cellulose (~30–50% by weight), hemicellulose (~19–45% by weight), and lignin (~15–35% by weight) (Mood et al. 2013; Feng et al 2013). However, bioconversion of ligno-cellulosic biomass into by-products is usually bottlenecked, as a result of biomass recalcitrance that prevents effective bioconversion due to the highly ordered, inaccessible crystalline regions present in ligno-cellulosic biomass many of which, are hydrophobic in nature (Gao et al, 2013). For catalysis to proceed, the bottleneck imposed by the inaccessible regions must be overcome hence, pre-treatment procedures have been utilized to help improve the surface area of exposure during hydrolysis ligno-cellulosic biomass (Arantes and Saddler 2011; Gao et al., 2013). In anaerobic digestion of ligno-cellulosic biomass, mechanical treatment using ball milling has been utilized to improve the surface area of these biomass. Mashandete et al., (2006) and Chandra et al (2015) have reported improve yield in biogas production via particle size reduction.

Anaerobic digestion of ligno-cellulosic biomass into biogas is a multi-step reaction in which, the hydrolytic step is identified as the first and rate limiting step (Pavlostathis and Giraldo Gomez, 1991; Vavilin et al., 2008; Tomei et al., 2008; Noike et al, 1985; Momoh and Nwaogazie 2011). The hydrolysis step has been described by Miron et al (2000) and Garala et al, (2003) as a complicated step, usually involving the initial adsorption and colonization of hydrolytic bacteria on surface of the ligno-cellulosic biomass (Vavillin et al 2008) after which, these hydrolytic bacteria subsequently release a suite of exo-enzymes called cellulase from a multi-enzyme complex known as cellulosome (Artzi et al, 2017). In the second step, the individual cellulose chains are disintegrated from the surface by sliding of cellulose chain into the catalytic site of cellulase leading to the formation of enzyme substrate complex (Gao et al 2013). Finally, the process proceeds to produce cellobiose or

other glucose monomers and by-products (Gao et al 2013). However, the efficiency of the cellulase activity is seriously handicapped by factors related to the ligno-cellulose structure and attempt to understand the actual factors responsible for the inefficient bioconversion of ligno-cellulosic biomass to biofuels has been blamed on a wide array of factors that include; substrate-related factors, enzyme-related factors and fractal-like-related factors (Bansal et al., 2009). The substrate-related factors relates to the physical ultra-structure and chemical nature of ligno-cellulose. Some examples of physical ultra-structure affecting ligno-cellulose hydrolysis includes; crystallinity, porosity, surface area, particle size and shape, pore volume, and degree of polymerization (Taherzadeh and Karimi 2008; Zhao et al., 2012) while, factors related to the chemical nature of ligno-cellulose biomass includes the presence of lignin, hemicellulose and acetyl group presence (Zhao et al., 2012). More recently, the hydrogen bonding arrangement in cellulose has also observed to influence rate of ligno-cellulose hydrolysis (Zhao et al., 2012; Gao et al., 2013). Apart from the substrate-related factors, enzyme-related factors have been observed to affect hydrolysis rate. For example, Fernandez et al, (2001) reported a relatively high hydrolysis rate in anaerobic biodegradability tests with high inoculum-to-substrate ratio indicative of some degree dependence on hydrolytic bacteria/enzyme concentration. Other factors such as, enzyme source or inoculum history, by-product inhibition by cellulose, and non-productive binding (NPB) of enzyme by lignin have been linked to enzyme-related factors affecting ligno-cellulose hydrolysis (Yang et al. 2011, Gao et al 2013).

More recently however, fractal-like-related factors have been associated with inefficient bioconversion of ligno-cellulose into by-products (Bansal et al., 2009). Fractal-like-related factors consider the reaction between ligno-cellulosic biomass and cellulase enzyme as a typical heterogeneous reaction in which, the reaction medium consists of a solid and liquid phase respectively (Xu and Ding, 2007). After adsorption of cellulase onto the solid surface of ligno-cellulose biomass, the cellulase diffuses on the surface in order to reach the active site for catalysis to occur. However, the presence of non-reactive and inaccessible regions on ligno-cellulosic biomass can act as obstacle that slows down diffusion of cellulase thus, inducing fractal-like hydrolysis kinetics or anomalous diffusion kinetics (Bansal, et al., 2009). Berry (2002), showed that increase in obstacle density leads to increase in fractal-like behaviour and this phenomenon can result in the clustering of diffusing enzymes on surface irregularities, making them spatially or topologically constrained thus, encouraging reactant segregation (Congcong et al, 2015; Bansal et al, 2009). In essence, the presence of non-reactive and inaccessible regions on ligno-cellulose biomass can give rise to non-uniform mixing and slow catalytic rate because, the frequency of fruitful collision with the active sites is significantly reduced (Bansal et al., 2009). In general, the inefficiency of the

cellulase catalytic process may not only be limited to the physical/chemical nature of the ligno-cellulosic biomass, the nature of enzyme and fractal-like-related factors also contribute in affecting lignocellulose hydrolysis (Bansal et al, 2009).

Mathematical approach into understanding the kinetics of hydrolysis and biogas production kinetics abound in literature. Generally, kinetic models for studying ligno-cellulose degradation can be classified into the following: (i) Firstly, those that depend on enzyme concentration and substrate concentration. These models which comprised mainly of the Michaelis-Menten-based substrate utilization models have been utilized by authors such as, Carlos et al. (2014) and Bezerra and Dias (2004) for studying hydrolysis of cellulosic biomass. However, a lot of reservation has accompanied this approach due to the following reasons that include; (a) Michaelis-Menten equation was developed under quasi-steady state assumptions in which an excess substrate to enzyme ratio was utilized. This is not achievable for ligno-cellulose biomass where the fraction accessible to enzyme range from 0.002 – 0.04 (Hong et al 2007; Bansal et al., 2009). (b) Michaelis-Menten equation was originally developed for homogenous conditions and hence, cannot be applied directly to heterogeneous reactions of enzymatic hydrolysis of insoluble substrate (Bansal et al., 2009). (ii) Secondly, those that depend on bacteria population and substrate concentration such as the Monod's based substrate utilization models (Tomei et al., 2008; Vavillin et al., 2008). (iii) Thirdly, those that depend on physical properties of substrate and substrate concentration and/or bacterial biomass concentration (Vavillin et al., 2008; Sanders 2000; Esposito et al 2008; Terashima and Lin (2000). (iv) Fourthly, those that are biomass independent in which hydrolysis was approximated as a zero or first-order process. In this approach, the bacteria growth or enzyme concentration are insignificant (Eastman and Ferguson, 1981; Schwarz 2001; Lynd et al., 2002; Rotter et al., 2008; Manfred et al., 2015) leading to oversimplification of the very complex process of hydrolysis.

More recently however, models that considered the heterogeneous nature of the medium have emerged (Valjamae et al., 2003; Xu and Feng 2009). This model largely classified as fractal-like kinetic are modifications of the first-order kinetic equations (Valjamae et al., 2003). Also, Xu et al., (2007), incorporated the fractal exponent (h) into the Michaelis-Menten kinetic equation in order to explain the phenomenon of overcrowding of cellulase onto cellulose surface. The idea of fractal kinetics was first proposed by Kopelman (1986) to describe reactions occurring under spatially constrained or topologically constrained environment (Berry, 2002; Bansal et al, 2009; Kopelman, 1986). Fractal kinetics rate coefficients have been shown to exhibit temporal time dependence and as such, the rate coefficient (k_t) in topologically constrained environment is re-expressed in a time

dependent formalism written as, $k_{(t)} = k_o t^{-h}$, with $0 \leq h \leq 1$ for $t > 0$ (Kopelman, 1988). In this expression, k_o represents the rate constant in ideal homogenous conditions, while (h) represent the fractal exponent (Berry, 2002; Bansal et al, 2009; Kopelman, 1986). However, this fractal like formalism suffers the problem of stability because it breakdown as $t \rightarrow 0$ for $h > 0$ (Schnell and Turner, 2004). This problem led Schnell and Turner (2004) to develop another expression for the rate coefficient written as follows, $k_{(t)} = k^* (t + \tau)^{-h}$, where, k^* represents the rate constant for ideal homogenous solution, and τ represents the critical time when the reaction rate is driven by forces of over-crowding with $t \geq 0$ for values of h ranging from $0 \leq h \leq 1$ (Schnell and Turner, 2004). In addition, other modification has been proposed by the authors such as Sadana (2001) and Varju et al, (2014) in which $k_{(t)} = k' (t+1)^{-h}$ with $t \geq 0$ for values of (h) ranging from $0 \leq h \leq 1$.

This work attempts to develop an inclusive mechanistic kinetic model, capable of studying effect of various particle size fractions on hydrolysis rate and biogas yield from anaerobic co-digestion of equal proportion of rice husk and cow dung. This study also attempts to evaluate the kinetic factors contributory to improved hydrolysis and biogas yield for smaller particle size fractions obtained from mechanical pre-treatment of ligno-cellulosic biomass.

2. Materials and Methodology

2.1 Experimental method

Rice husk was collected and washed to remove dirt, stones etc., and dried to 105°C to remove moisture. The dried rice husk was milled using a pulverizer (HR 2094, Philips made in Netherlands) and subsequently sieved to obtain particle sizes of different dimensions using British sieves corresponding to the following BS sieves of 100-70, 70-52, 52-25, 25-16, and 16-10 respectively. These sieves were able to yield particle size fractions of the following range: 0.150-0.212mm, 0.212-0.300mm, 0.300-0.600mm, 0.600-1.000mm and 1.000-1.700mm in mean diameter. The cow dung was obtained from abattoir situated in from abattoir situated at Choba community, Rivers state, Nigeria. The dung was air-dried until a constant weight was obtained. The stomach chambers of ruminant animals such as cattle, and other animals such as goat, sheep, horse, etc. are known to contain anaerobic microbes (Ranade et al., 1979) and as such, no addition of inoculum was required. According to Rastogi et al., (2008) methanogenic anaerobic bacteria in cow dung still retained their methanogenic activity even after air drying for more than two months, such that, air-dried cow dung was reported still suitable as substrate for biogas production with little loss in methane production.

The cow dung were subsequently pulverized and sieved as previously conducted for the rice husk to obtain particle size fractions of the following range: 0.150-0.212mm, 0.212-0.300mm, 0.300-0.600mm, 0.600-1.000mm and 1.000-1.700mm in mean diameter. Equal weight of the sieved fractions rice husk and cow dung were mixed together and allowed to undergo anaerobic digestion in batch digesters Labeled A-E (Table 2) after the analysis of their volatile solids contents and carbon to nitrogen ratio using standard methods (APHA, 1986). Weighing was conducted using weighing balance (Mettler, model PN163, manufactured in Switzerland with specification range between 0.10 mg and 500 g) and volatile solids determination was conducted using a muffle furnace (Carbolite model LMF 4, UK).

2.2 Experimental Procedure

The crushed rice husk and cow dung were mixed thoroughly in equal proportion and loaded into Buchner flasks of 500ml capacity containing 250ml of water. A uniform mixture of rice husk and cow dung mixture was achieved by proper mixing of both biomasses before loading into batch reactors. The flasks were labeled digester A, B, C, D and E which comprised particle size ranging from 0.150 - 0.212mm, 0.212 - 0.300mm, 0.300 - 0.600mm, 0.600 - 1.000mm and 1.000 - 1.700mm respectively. Total solids (TS) were fixed at 8.5% (w/w) in 250ml of water which was within the recommended range of 4-12% suggested by Tchobanglous et al (1993) for low solids loading and experiments were conducted in duplicates (A-1, B-2, C-3, D-4 and E-5). Also, duplicate runs were conducted for another set of digesters labelled F, G, H, I and J comprising similar solids compositions of cow dung and rice husk mixture, however, the cow dung was autoclave before mixing with the rice husk that served as the control digesters, (F-1, G-2, H-3, I-4 and J-5). The digesters were corked to exclude air and allowed to run at ambient temperature with mean value of $30 \pm 3^{\circ}\text{C}$ (which was within the mesophilic temperature range) for 75 days (which comprised days of active decomposition and biogas production) while, the contents of the digesters were shaken twice daily i.e., morning and evening. Biogas produced was collected by saturated brine water displacement method (Momoh and Nwaogazie, 2008). The saturated brine solution prevented the dissolution of carbondioxide in water. The characteristics of feedstock and compositions of digesters A-E are as shown in Table 1-2.

Fig. 1: Flow sheet on experimental methodology

Table 1: Characteristic of feedstock

Feedstock	Ash Content (%)	Volatile Solids (%)	Carbon/Nitrogen
Cow dung	29.20	70.80	17:1
Rice Husk	15.77	84.23	33:1

Table 2: Digester Solids Composition

Digester	Particle size (mm)	Rice husk (g)	Cow dung (g)	Total Solids (%)	Volatile solids concentration (g/L)	Carbon / Nitrogen ratio (C/N)
A	0.150-0.212	11.6	11.6	8.5	72.0	25:1
B	0.212-0.300	11.6	11.6	8.5	72.0	25:1
C	0.300-0.600	11.6	11.6	8.5	72.0	25:1
D	0.600-1.000	11.6	11.6	8.5	72.0	25:1
E	1.000-1.700	11.6	11.6	8.5	72.0	25:1

3. Theory/Calculation

This study attempts to elucidate the kinetics of hydrolysis and biogas production from complex biomass taking into cognizance factors such as; (i) Adsorption for enzyme/bacterial biomass on ligno-cellulosic biomass (ii) hydrolytic bacterial/enzymatic catalytic rate (iii) Heterogeneous or fractal nature of environment. The adsorption of enzyme/bacteria biomass onto the ligno-cellulosic biomass was utilized to evaluate the level inaccessible regions on ligno-cellulosic biomass while, successful catalysis leading to biogas production was assessed by a hydrolysis rate component. Lastly, the heterogeneous nature of the medium was followed by the fractal exponent (h).

The model development process involved modifying the Monod's substrate utilization models (Momoh et al, 2013) which is written as follows,

$$-\frac{dS}{dt} = \frac{\mu_m}{Y} \left(\frac{SX}{K_s + S} \right) \quad (1)$$

Where X represents the hydrolytic bacteria population (g/L), S represents the volatile solids concentration (g/L), K_s represents the half saturation constant (g/L) and $-\frac{dS}{dt}$ represents the rate substrate utilization by the hydrolytic bacteria population. The hydrolytic bacteria yield coefficient

was represented by (Y) while the maximum specific growth rate was represented by μ_m (day^{-1}). Because, only a small fraction of cellulose is accessible to the hydrolytic bacteria population in the range of 0.002 – 0.04 (Hong et al 2007; Bansal et al 2009), it becomes possible to express Eq. (1) by the expression given as,

$$-\frac{dS}{dt} = \left(\frac{SX}{Y} \right) \left(\frac{\mu_m}{K_s} \right) \quad (2)$$

Where $\frac{\mu_m}{K_s}$ represented the specific affinity (L/g/day) (Kavarova-Kovar and Egli, 1998) of hydrolytic bacteria for lignocellulose biomass. This rate constant can be view as a dynamic parameter that is time dependent due to medium heterogeneity. Heterogeneity arises due to the presence of irregularities, non-reactive or inaccessible regions present on ligno-cellulosic biomass. These irregularities can contribute to varying rate of adsorption of hydrolytic bacteria/enzymes onto the surface of the biomass with time. Hence, a time dependent coefficient following the time dependent formalism of Sadana (2001) and Varju et al, (2014) was utilized in this study to represent the dynamic nature of adsorption. Thus, the specific affinity was represented by the expression,

$$\frac{\mu_m}{K_s} = k'_o (t + 1)^{-h} \quad t \geq 0 ; \quad 0 \leq h \leq 1 \quad (3)$$

Where k'_o represents the specific affinity (L/g/day) in ideal homogenous medium and term (h) represents the fractal exponent. However the yield coefficient (Momoh et al, 2013) can be expressed as,

$$\frac{dX}{dS} = Y \quad (4)$$

Such that,

$$X = (Y(S_o - S) + X_o) \quad (5)$$

Substituting Eq. (4) and (5) into Eq. (2) gives,

$$-\frac{dS}{dt} = k'_o (t + 1)^{-h} \left(\frac{Y(S_o - S) + X_o}{Y} \right) S \quad (6)$$

This can be re-arranged as,

$$\frac{dS}{dt} = (t + 1)^{-h} \left(k'_o S^2 - S \left(S_o k'_o + \frac{X_o}{Y} k'_o \right) \right) \quad (7)$$

Eq. (7) can be reduced to,

$$\frac{dS}{dt} = (t + 1)^{-h} (k'_o S^2 - S(k'_o S_o + b)) \quad (8)$$

353 Where $b = \frac{X_o}{Y} k'_o$

354

355 However, Eq. (8) can further be reduced to,

$$356 \quad \frac{dS}{dt} = (t+1)^{-h} (k'_o S^2 - dS) \quad (9)$$

357 Where $d = (k'_o S_o + b)$

358 Where S_o , represents the initial volatile solids concentration (g/L) and (t) represents time (days).

359 Eq. (9) can be solved by integration using the partial fraction approach. Thus, by expressing Eq. (9)
360 in partial fractions terms one obtains,

$$361 \quad \int \left(\frac{A}{S} + \frac{B}{k'_o S - d} \right) dS = \int (t+1)^{-h} dt \quad (10)$$

362 The constants A and B can be deduced upon expansion of the left hand term of Eq. (10) which gives,

$$363 \quad A(k'_o S - d) + BS = 1 \quad (11)$$

364 Collecting appropriate coefficients yields $A = -\left(\frac{1}{d}\right)$ and $B = \left(\frac{k'_o}{d}\right)$

365 Thus, substituting for A and B in Eq. (10) yields,

$$366 \quad \int \frac{-1}{dS} + \frac{k'_o}{d(k'_o S - d)} = \int (t+1)^{-h} dt \quad (12)$$

367 Upon integrating one obtains,

$$368 \quad \ln \left(\left(\frac{k'_o S - d}{k'_o S_o - d} \right) \times \frac{S_o}{S} \right) = d \left(\frac{(t+1)^{1-h} - 1}{1-h} \right) \quad (13)$$

369 Such that,

$$370 \quad S = \frac{dS_o}{k'_o S_o - (k'_o S_o - d) \exp \left(d \left(\frac{(t+1)^{1-h} - 1}{1-h} \right) \right)} \quad (14)$$

371 Using the correlation as utilized by Linke (2009) to relate between biogas yield and substrate given
372 by Eq. (15)

$$373 \quad \frac{y_m - y_t}{y_m} = \frac{S}{S_o} \quad (15)$$

374 Where y_m and y_t represent the maximum biogas yield and biogas yield at time (t) respectively,
375 expressed L/g volatile solids.

376 Hence, upon substituting Eq. (15) into Equation (14) one obtains,

$$y_t = y_m - \frac{y_m d}{k'_o S_o - (k'_o S_o - d) \exp \left(d \left(\frac{(t+1)^{1-h} - 1}{1-h} \right) \right)} \quad (16)$$

Substituting $d = (k'_o S_o + b)$ in Eq. (16) one obtains,

$$y_t = y_m - \frac{y_m (k'_o S_o + b)}{k'_o S_o + b \exp \left((k'_o S_o + b) \left(\frac{(t+1)^{1-h} - 1}{1-h} \right) \right)} \quad (17)$$

The model developed in this study can be viewed as an aggressive attempt to incorporate the various factors affecting lignocellulose hydrolysis into a simplified expression with three dominant components. The biogas yield was preferred for modelling purpose over the methane yield as utilized by Linke (2006) because the product of hydrolysis which is glucose ultimately converts into carbondioxide and methane. The term k'_o which represents the specific affinity of hydrolytic bacteria/enzyme for substrate in ideal homogenous conditions. It indirectly indexes the physical and chemical related factor affecting ligno-cellulose hydrolysis. The physical structures affecting ligno-cellulose hydrolysis includes; crystallinity, porosity, surface area, particle size and shape, pore volume, and degree of polymerization (Zhao et al., 2012) while, factors related to the chemical nature of ligno-cellulose biomass include, the presence of lignin, hemicellulose and acetyl group presence and hydrogen bonding arrangements (Zhao et al., 2012). This term k'_o would subsequently be referred to as specific affinity for purposes of ease. A high value for the specific affinity is indicative of the fact that, the physical structure and chemical nature of the ligno-cellulosic biomass encourages high binding of hydrolytic bacteria/enzymes to ligno-cellulosic biomass, while low value of this parameter is indicative of the fact that, the physical structure and chemical nature of ligno-cellulose encourages low binding of hydrolytic bacteria/enzymes to ligno-cellulosic biomass.

The term $b = \frac{X_o}{Y} k'_o$ represents the initial hydrolysis rate (day^{-1}). This parameter indexes the initial hydrolytic bacteria/enzyme related factors which affects ligno-cellulose hydrolysis. The higher the value of this parameter, the higher the initial hydrolysis rate and vice-versa. This parameter is of great importance in evaluating slowed diffusion of cellulase on ligno-cellulosic biomass. Finally, the term “ h ” represents the fractal exponent and it relates to the fractal nature of the medium. High value of this parameter, implies high degree of obstacle presence in the medium and vice-versa. Thus, obstacles in the form of inaccessible regions in the medium can be indicative or contributory to higher fractal exponents. In addition, this fractal-like kinetic model has the ability to predict possible

rate limiting step of biodegradation processes. For example, if the difference between the overall affinity and the initial hydrolysis rate ($k'_o S_o - b$) is a positive value, then hydrolysis is rate limiting. However, if the difference is negative, then hydrolysis may not be rate limiting for the degradation process.

4.0 Results and discussion

In this research work, a simplified experimental study was carried out to assess the effect of particle size fractions obtained from milling on hydrolysis rate and biogas yield during co-digestion of equal proportion of rice husk and cow dung. The solid loading was fixed at 72g VS/L while the experiment was conducted at ambient conditions in batch digesters. The volatile solids contents of the rice husk and cow dung were determined to be 84.23% and 70.80 % respectively of their corresponding dry solids while the C/N ratio were 33:1 and 17:1 for the rice husk and cow dung respectively. These values are close to that reported by Kumar et al (2010) and Matheri et al., (2015) for C/N ratio for rice husk and cow dung respectively. The mixture of rice husk and cow dung, with particle size fractions: 0.150 - 0.212mm, 0.212 - 0.300mm, 0.300-0.600mm, 0.600-1.000mm and 1.000-1.700mm were loaded into digesters labelled A, B, C, D and E respectively with an average C/N ratio of 25:1. After an active decomposition period of 75days, the experimental (exp.) cumulative biogas yield was observed to increase as particle size fractions decreased, with digester A having the smallest particle size fractions producing the highest cumulative biogas yield of 0.156 ± 0.007 L/g VS while, digester E with the largest particle size fractions produced the least cumulative biogas yield of 0.082 ± 0.008 L/g VS (Table 3). In essence, cumulative biogas yield was observed to be inversely proportional to particle size (Fig. 3, 4 and 5). This finding is consistent with previous works Hills and Nakano (1984) and Sharma et al., (1988) who observed that a linear relationship existed between the biogas production and the inverse of the particle diameter. According to Arantes and Saddler (2011), an inverse relationship can be established between average particle size of ligno-cellulose biomass and increase accessible area. The reason for higher biogas yield as particle size gets smaller has been anchored on the premise of “increased surface area” of exposure associated with smaller particles (Arantes and Saddler, 2011).

For the controlled experiments the solid loading was fixed at 8.5% comprising varying particles size fractions of cow dung and rice husk. The biogas production from the controlled experiments was very insignificant in all digesters labelled F, G, H, I and J and the duplicates. This clearly show the role micro-organisms play in the hydrolysis and bio-gasification of these substrates, and that cow dung can serve both as co-substrate and also, a source of inoculum for the experiment.

4.1 Effect of particle size on hydrolysis and biogas yield kinetics

The model developed in the study identified three parameters that may be important in regulating hydrolysis on ligno-cellulosic biomass, that include, (i) the specific affinity k'_o with units of L/g/day

(ii) the initial hydrolysis rate $\frac{X_o}{Y}k'_o$ (which is, the product of the specific affinity and a term $\frac{X_o}{Y}$)

(iii) The fractal exponent (h). In order to determine these parameters, a parameter estimation exercise was conducted using non-linear regression technique via solver function option of the Microsoft Excel toolpak. The results for the parameter estimation are as shown in Table 3. In addition, the level of interrelationship between these parameter where evaluated using the principal component analysis (PCA) technique, which was conducted using XLSTAT (2014 version) add-ins option of the Microsoft Excel toolpak and presented in Table 4 and Fig. 2.

Table 3: Exp. Biogas yield and Parameter Estimation for the fractal-like kinetic model

Digester	Particle size (mm)	Exp. Biogas yield (L/gVS)	Parameter Estimation for the fractal-like kinetic model					
			y_m (L/gVS)	k'_o (l/g/day)	$b = \frac{X_o}{Y}k'_o$ (day ⁻¹)	h	r	RMSE
A	0.150-0.212	0.156 ± 0.007	0.1844	0.002468	1.35E-03	0.222	0.996	0.004218
B	0.212-0.300	0.130 ± 0.008	0.1530	0.002346	1.36E-03	0.210	0.996	0.00351
C	0.300-0.600	0.105 ± 0.005	0.112	0.006003	8.35E-04	0.404	0.998	0.002404
D	0.600-1.000	0.085 ± 0.009	0.0932	0.073873	1.48E-06	1.140	0.998	0.001724
E	1.000-1.700	0.082 ± 0.008	0.0837	0.071189	6.45E-07	1.090	0.997	0.002104

4.1.1 Assessment medium heterogeneity (fractality) on the kinetics of biogas production

In this study, cumulative biogas yield was observed to be inversely proportional to particle size that is, the highest total amount of biogas yield corresponded with the smallest particle size fractions as shown in Fig. 3, 4 and 5. Milling has been reported to improve biomass susceptibility to enzymatic hydrolysis because, it does not only reduce particle size, but also reduces ligno-cellulose crystallinity, increase pore size and pore volume (Zhao, 2008; Taherzadeh and Karimi et al, 2008). Hence, the direct attribution of particle size to increase biomass conversion has been challenged. According to Taherzadeh and Karimi et al, (2008), the term accessible surface area should be considered as an “individual factor” that affects hydrolysis of ligno-cellulosic biomass. This term was suggested to comprise all other factors such as crystallinity, pore size, pore volume, particle size etc. Thus, the

action of milling in producing smaller particle sizes could be properly viewed as increasing the accessible surface area of ligno-cellulose feedstock.

The reduction in the degree of inaccessible regions as particle size decreased was confirmed from kinetic studies conducted in this research. The term called the fractal exponent (h) which measure the degree of heterogeneity, obstacle presence, inaccessible regions or recalcitrancy present in the medium was observe to increase as particle size fraction increased as shown in Fig. 3. It was observed that, particle size fractions ranging from 0.15 - 0.225 mm, 0.225 - 0.3 mm and 0.3 - 0.6 mm were less heterogeneous than particle size fractions ranging from 0.6 - 1mm and 1 -1.7mm. This finding was further corroborated from principal component analysis (PCA) which showed that particle size fractions ranging from 0.15 - 0.225, 0.225 - 0.3 and 0.3 - 0.6 mm completely laid on opposite plane to particle size fractions ranging from 0.6 - 1 and 1 -1.7mm. Hence, because higher fractal exponent (h) are associated with particle size fractions ranging from 0.6 - 1 and 1 -1.7mm, they were considered more heterogeneous in nature with more obstacles, inaccessible regions or recalcitrancy as compared to the lower fractal exponents associated with particle sizes fraction ranging from 0.15 - 0.225, 0.225 - 0.3 and 0.3 - 0.6 mm. In general, milling result in smaller particle size fractions which possess less inaccessible regions that encouraged hydrolytic process and more biogas production whereas, larger particle size fractions seemed to have more inaccessible regions or obstacles presence that discouraged hydrolytic process and reduce biogas production as shown in Fig. 3. In a similar vein, Liew et al., (2012) has previously reported an inverse linear relationship between the methane yield and the lignin content (obstacle presence).

Fig.2: Biplot for interrelationship between estimated parameters

Table

Factor loadings:	F1
biogas yield	0.924
spec. affinity	-0.970
Initial hydr. Rate	0.997
fractal exp. (h)	-0.991

4: loading factor for various estimated parameters

Fig. 3: Effect of particle size fractions on fractal exponent and biogas yield

4.1.2 Assessment of specific affinity on biogas yield

The specific affinity which is a measure of the binding of hydrolytic bacteria/enzyme on ligno-cellulosic biomass was estimated as shown in Table 3. Also, Fig. 4 shows the relationship between this parameter and biogas yield for different particle size fractions. Particle size fractions ranging from 0.15 - 0.225, 0.225 - 0.3 and 0.3 - 0.6 mm showed low affinity for hydrolytic bacteria/enzymes, while particle size fractions ranging from 0.6 - 1 and 1 - 1.7 mm showed higher affinity for hydrolytic bacteria/enzyme (Fig. 4). In essence, smaller particles sizes fractions, with lower degree of fractality, less obstacle presence or recalcitrancy, seemed to show less binding compared to the larger particle size fractions that were identified with higher fractal exponents. Thus, higher obstacle presence or inaccessible regions in ligno-cellulose biomass may lead to strong binding attachment of hydrolytic bacteria or enzymes and vice versa. In essence, the increase biogas yield observed at lower particle size could be attributed to lower affinity of hydrolytic bacteria/enzyme towards these particle sizes whereas, decreased biogas yield observed for larger particle sizes fractions could be attributed to stronger affinity for hydrolytic enzymes/bacteria. Nakamura et al (2014), described this phenomenon as a trade-off that usually exists in the hydrolysis of cellulosic biomass by cellulase, where weak binding encourages easy dissociation of cellulase enzyme for fast catalysis.

Moreover, principal component analysis (PCA) revealed that, the specific affinity which was observed with loading factor of -0.97 (Table 4) is a strong factor to consider when dealing with larger particle size fractions ranging from 0.6 - 1.0 and 1.0 - 1.7 mm (Fig. 2). These larger size fractions were also shown to be characterized by high fractal exponents as revealed by the principal component analysis (PCA) with loading factor of -0.986 (Table 4). This, implies that higher inaccessible regions or obstacle presence in the ligno-cellulosic biomass would always correlate with high specific affinity. This findings aligns with the statements of Nakamura et al (2014) who concluded that high affinity was advantageous for hydrolysis of crystalline cellulose or more recalcitrant cellulose.

Fig. 4: Effect of particle size fractions on specific affinity and biogas yield

4.1.3 Assessment of initial hydrolysis rate and biogas yield

The initial hydrolysis rate, $\frac{X_o}{Y} k'_o$ is a measure of the catalytic efficiency of the hydrolytic bacteria/enzyme. It represents the capacity of the hydrolytic enzyme to disintegrate and diffuse to process cellulose micro-fibrils into by-products. The presence of inaccessible regions or obstacle presence on ligno-cellulose biomass would invariably slow down the diffusion, disintegration and processive action of hydrolytic biomass. The behaviour of this parameter is captured in Fig. 5, where smaller particle size fractions resulted in higher initial hydrolytic rates and higher biogas yield, while large particle size fractions were associated with lower initial hydrolytic rates and lower biogas yield. Particle size fractions ranging from 0.15 - 0.225, 0.225 - 0.3 and 0.3 - 0.6 mm led to increased initial hydrolysis rates compared to particle size fractions ranging from 0.6 - 1 and 1.0 - 1.7 mm which showed lower initial hydrolysis rate (Fig. 5).

Also, results from principal component analysis (PCA) (Fig. 2) revealed that the initial hydrolysis rate is a principal factor when considering hydrolysis of smaller particle size fractions ranging from 0.15 - 0.225, 0.225 - 0.3 and 0.3 - 0.6 mm. This initial hydrolysis rate was observed with a loading factor of 0.9943 (Table 4) which correlated positively with biogas yield with a loading factor of 0.9234 (Table 4). This implies that for improvement in biogas yield, the initial hydrolysis rate must be appreciably high.

Fig. 5: Effect of particle size fractions on initial hydrolysis rate and biogas yield

Again, this finding is consistent with recent report by Nakamura et al (2014), who concluded that high hydrolytic activity (rates) characterized by higher dissociation rate constants (low affinity) is predominant for the degradation of the amorphous cellulose region, less recalcitrant cellulosic or easily accessible biomass. This result supports our earlier claim that increasing particle size tend to correspond with increase in the degree of inaccessible regions, which can act as obstacle to the free movement of hydrolytic enzymes during hydrolysis of ligno-cellulosic micro-fibrils.

4.2 Inaccessible regions: A bane to efficient ligno-cellulose hydrolysis and biogas production

The hydrolysis of ligno-cellulosic biomass into reducing sugars prior to anaerobic conversion to biogas remains a complicated subject with researcher sometimes conflicting on the actual factor responsible for fast and slow behaviour of ligno-cellulose hydrolysis (Taherzadeh and Karimi et al, 2008; Arantes and Saddler, 2013). While some researchers has blamed it solely on the crystallinity index (Hall et al 2010), others has suggested the pore size, pore volume (Chandra et al, 2007; Lyand and Lynd, 2004; Arantes and Saddler, 2011), while some has blamed lignin and hemicellulose presence (Mansfield et al 199; Bansal et al 2009; Zhao et al., 2013) as the major factor affecting (ligno)-cellulosic biomass hydrolysis.

Ligno-cellulosic biomass are known to comprise both crystalline and amorphous regions, with the crystalline region comprising about 2/3 of the total cellulose which is in the crystalline form (Chum et al., 1985). Cellulase readily hydrolyzes the more accessible amorphous portion of cellulose, while

the enzyme is not so effective in degrading the less accessible crystalline portion hence, it is widely expected that high-crystallinity cellulose will be more resistant to enzymatic hydrolysis, and that decreasing the crystallinity would increase the digestibility of lignocelluloses. On contrary, some studies (Wyman 1996) have shown more digestibility for more crystalline lignocelluloses. According to Taherzadeh and Karimi et al, (2008), this conflict in the reports might continue to appear, as long as other factors, which contributes to increased accessible surface area, such as, reduced particle size, high porosity and pore volume, reduced degree of polymerization (DP), reduced lignin content and nature of hydrogen bond interactions etc., are ignored. Hence, blaming any one of these factors as solely responsible for the fast and slow hydrolytic behaviour associated with the hydrolysis ligno-cellulose would definitely lead to contradictory outcomes.

This study thus suggests, the use of the term “inaccessible regions” present on ligno-cellulosic biomass as a more encompassing factor responsible for slowdown kinetics of ligno-cellulose hydrolysis, since what comprise inaccessible regions on ligno-cellulosic biomass could be a plethora of factors ranging from high crystallinity, high lignin, hemicellulose and acetyl group presence, high degree of polymerization, low specific surface area (Bansal et al 2009), and more recently the nature of hydrogen bond interactions (Gao et al 2013; and Zhao et al 2012). These inaccessible regions can easily make these biomass largely hydrophobic in nature. Thus, attempting to understand the mechanism of hydrolysis of ligno-cellulose biomass without consideration of all these factors would lead to inconclusive findings and any process that will require the monitoring of all these factors would definitely involve extensive and expensive research techniques. For simplicity, fractal exponent (h) can be utilized to index the degree of inaccessible regions in ligno-cellulosic biomass.

The consequence of inaccessible regions have been reported to induce slowdown kinetics, leading to anomalous diffusion or fractal-like kinetics (Bansal et 2009; Kopelman, 1988). In addition, at very high obstacle density, not only do slowdown of enzyme occurs, but also, enzyme become trapped or sequestered around these obstacles reducing their catalytic rate and availability (Tremmel et al., 2007; Mourao et al 2014). This phenomenon are sometimes referred to as traffic jam condition (Igarashi et al 2011). In this study therefore, the consequences of obstacle presence or inaccessible regions in slowing down hydrolysis rate and/or sequestration of hydrolytic enzymes during ligno-cellulose hydrolysis seemed to have been captured. It was observed that as particle size fractions increased from 0.15 - 0.225mm to 0.3 - 0.6 mm, the initial hydrolysis rate gradually decreased from 0.002 day^{-1} to 0.001 day^{-1} (Fig. 5) with a corresponding increase in the fractal exponent from 0.22 to 0.414 (Fig. 3). These observation is a clear representation of slowed down diffusion as particle size

fractions increased within this particle size range. However, a significant drop in initial hydrolysis rate and also, biogas yield was observed as particle size fractions increased from 0.6 - 1mm to 1.0 - 1.7mm. The initial hydrolytic rates were observed to be $2.3\text{E-}05 \text{ day}^{-1}$ and $3\text{E-}06 \text{ day}^{-1}$ respectively (Fig. 5), while the fractal exponents were observed to be 1.13 and 1.09 respectively (Fig. 3). This very low values for the initial hydrolysis rate seem to be indicative of trapping, sequestration or high traffic jamming conditions of hydrolytic enzyme resulting in non-productive binding (NPB), which is peculiar at very high obstacle presence or inaccessible regions on ligno-cellulosic biomass. This situation can also lead to non-availability of hydrolytic enzyme as the obstacle density further increases, which may shift reaction kinetics from been fractal-like diffusion limited to rather reaction limited conditions at very high obstacle presence (Tremmel et al., 2007; Maurou et al 2014).

Although, the larger particle sizes fractions ranging from 0.6 – 1.0 mm and 1.0 - 1.7mm yielded fractal exponent that exceeded the limit set for fractal like kinetics that is, $0 \leq h \leq 1$, such observations are not unusual. According to Wang and Feng (2011), such case do represent evidence of irreversible binding of enzyme to high lignin content during ligno-cellulose hydrolysis leading to non-productive binding that diminishes enzyme bio-availability. In a similar vien, Mourao et al. (2014), reported that very high obstacle density in medium has the potential to sequester enzyme molecules contributing to diminishing availability of enzyme and slower catalytic process. Our findings thus seem to support all these claims, in which obstacle presence or inaccessible regions in ligno-cellulosic biomass may not only slow down hydrolytic enzyme diffusion but also, strongly bind, trap or sequester hydrolytic enzymes, which consequently lead to reduced hydrolysis rate and lower biogas yield.

4.3 Understanding the kinetic mechanism of ligno-cellulosic hydrolysis

The fractal-like kinetic model developed in this study seemed to show great potential in the unraveling the hydrolytic kinetic mechanism of ligno-cellulosic biomass due to, its ability to indirectly index the physical, fractal, chemical nature of ligno-cellulose biomass and also, the hydrolytic enzyme-related factors involved in hydrolysis. In a recent study conducted by Gao et al., (2013) to elucidate the mechanism of cellulose hydrolysis, they observed that strong binding or high affinity to cellulosic biomass may not be necessary for efficient hydrolysis of cellulose. By altering the crystalline structure of cellulose from its native allomorph I_{β} to cellulose III, they observed a 40–50% lower binding partition coefficient for fungal cellulase, but surprisingly, it enhanced hydrolytic activity on cellulose III. Interestingly however, the native allomorph I_{β} and cellulose III showed comparable crystallinity indices, specific surface area and similar available chain reducing ends. The

reason for reduced effective binding of cellulase to cellulose III was found to be related to the relatively lower hydrophobicity of the cellulose III fibril surfaces due to the nature of hydrogen bond arrangement. In addition, the reduced catalytic efficiency for the native allomorph I_β as compared to cellulose III was blamed on higher decrystallization energy due to the high hydrophobic nature of native allomorph I_β (Gao et al., 2013). Thus, in addition to binding of enzyme to exposed surface area, Gao et al., (2013) reported that another parameter called the k_{slide} may contribute to enzyme catalytic efficiency. This parameter was suggested to be responsible for disintegration and processivity of the individual cellulose micro-fibril. They observed that an increase in k_{slide} was coupled with a corresponding reduction in binding of cellulase on cellulose. In another corroborative research, Nakamura et al. (2014) using high speed atomic forced microscopy in studying cellulose hydrolysis reported that a trade-off exit between cellulase adsorption/binding on cellulosic biomass and its hydrolytic rate. They observed that weak binding between cellulase to substrate is always accompanied by high velocity of the cellulase enzyme and concluded that high affinity for cellulosic substrate reduces catalytic activity. In this study, by using a simplified kinetic model, a corroboration of the works of Gao et al (2013) and Nakamura et al. (2014) was observed in which, increase in specific affinity for ligno-cellulosic biomass was observed to correspond to decrease in hydrolytic efficiency and vice-versa (Fig. 4 and 5).

Critical examination of Eq. 17 revealed that, for efficient degradation of ligno-cellulosic biomass, two crucial intrinsic factors must be considered. First, the overall affinity of hydrolytic enzyme for ligno-cellulosic biomass ($k'_o S_o$) and secondly, the initial hydrolytic rate $\left(b = \frac{X_o}{Y} k'_o\right)$. This two factors are always in tandem, coupled together and working in opposite manner. High value of the overall affinity ($k'_o S_o$) simultaneously reduces the value of the initial hydrolytic rate $\left(b = \frac{X_o}{Y} k'_o\right)$ and vice-versa. For ligno-cellulose substrate with high obstacle presence or inaccessible regions, a high value would be expected for the overall affinity while, the initial hydrolysis rate would be expected to decrease (Table 3). However, for ligno-cellulose substrate with less inaccessible area or obstacle presence, a reduction in overall affinity is expected to be coupled with improvement in the value of the initial hydrolytic rate. Thus, the overall affinity rate of hydrolytic enzyme for ligno-cellulosic biomass ($k'_o S_o$) and the initial hydrolytic rate $(b) = \frac{X_o}{Y} k'_o$ may indirectly relate to the terms “binding partition coefficient and k_{slide} ” respectively as reported by Gao et al, (2013) and the terms “processivity and hydrolytic velocity” respectively as used by Nakamura et al., (2014). This finding

is very significant because proper control of the hydrolytic step can be conducted by acknowledging the presence of these two parameters. From our investigation, the initial hydrolytic rate $(b) = \frac{X_o}{Y} k'_o$ is always less than the overall affinity rate $(k'_o S_o)$ (Table 3). This is always the case for hydrolysis of ligno-cellulosic biomass otherwise, hydrolysis would not be the rate limiting step. Furthermore, because the initial hydrolysis rate is always lower compared to the overall affinity constant, it can be considered as the rate limiting step in hydrolysis of ligno-cellulosic biomass and the key towards improved hydrolysis and biogas production. This parameter which comprises of the specific affinity (k'_o) , the initial hydrolytic bacteria/enzyme concentration (X_o) and the ratio of changes in digested ligno-cellulosic biomass to change in bacteria concentration $\left(\frac{1}{Y} = \frac{\Delta S}{\Delta X}\right)$ bears close semblance to the description of the k_{slide} as reported by Gao et al, (2013). These authors describe this parameter as an important but ignored coarse-grained rate constant that influences intrinsic cellulase kinetics, which action may consists of multiple elementary steps, namely decrystallization of individual chains (breaking of hydrogen bonds and pulling out of chains) and enzyme procession (i.e., sliding of the chain into the enzyme active site by breaking interactions at current binding sites to move one glucosyl unit and reform interactions at new sites). Thus, by manipulating certain components of the initial hydrolysis rate such as, increasing the initial hydrolytic bacteria/enzyme concentration (X_o) , the initial hydrolysis rate may be increased for efficient hydrolysis and biogas production. This observation supports the conclusions of Gavala et al, (1999) who reported that the hydrolysis process is also very strongly dependent on the origin and previous acclimatization of anaerobic bacteria cultures. Also, this kinetic model explains why hydrolysis may not be rate limiting as observed by Fernandez et al, (2001) who reported a relatively high hydrolysis rate in anaerobic biodegradability tests with high inoculum-to-substrate ratio indicative of some degree dependence on hydrolytic bacteria/enzyme concentration. A very high inoculum- to - substrate ratio may sometimes allow the initial hydrolysis rate to be greater than the overall affinity for the given substrate shifting the rate limiting step from hydrolysis. In general, if the difference between the overall affinity and the initial hydrolysis rate $(k'_o S_o - b)$ is a positive value, then hydrolysis is rate limiting. However, if the difference is negative, then hydrolysis may not be rate limiting for that degradation process.

4.4 Model validation and comparison with the modified Gompertz equation for predicting biogas yield

The validation of the fractal-like model developed in this study was conducted by fitting the model estimated parameters against the experimentally observed biogas yield. The fractal-like model was observed to describe the experimental biogas yield with correlation coefficients (r) ranging between 0.996 – 0.998 and root mean square errors (RMSE) as shown in Table 3 and depicted by Fig. 6 -10. It implies therefore, that this 4-parameter model could be utilized for prediction of biogas yield from the co-digestion of rice-husk and cow dung and possibly other ligno-cellulosic biomass undergoing anaerobic digestion. The developed fractal-like model was further compared with another 3-parameter model utilized in the prediction of biogas production in batch reactors that is, the modified Gompertz equation. This equation has been utilized by althorns such as Lay et al., (1996), to study the cumulative methane production in biogas production. Recently, Budiyo et al., (2010) and Yusuf et al., (2008) have utilized this modified equation to described cumulative biogas yield. The modified Gompertz equation has enjoyed wide utilization and application despite its shortcoming of assuming a non-zero assumption for gas yield at initial time equals zero. In other words, a hypothetical initial gas volume was assumed at the start of digestion process (Scholfield et al. 1994). Other researchers such as, France et al., (2000) and Dijkstra et al., (2005) have criticize this flaw associated with the development of the modified Gompertz equation. However, despite this inherent error, its high predictive ability and meaningful biological parameter has popularize the utilization of this model. The modified Gompertz equation can be represented as,

$$y = y_m \exp \left(- \exp \left(\frac{R_m \times e}{y_m} (\lambda - t) + 1 \right) \right) \quad (18)$$

where, y represents the cumulative of biogas produced (L/g VS) at any time (t), (y_m) represents the maximum biogas production potential (ml), (R_m) represents the maximum biogas production rate (L/gVS/day) while, λ represents the lag phase (days), which is the minimum time taken to produce biogas or time taken for bacteria to acclimatize to the environment in days. The term e is equivalent to 2.71. The constants y_m , R_m , and λ were determined using non-linear regression approach with the aid of the solver function of the Microsoft Excel tool Pak and the results for the parameter estimates are shown in Table 5.

Table 5: Parameter Estimation for the Modified Gompertz kinetic model

Digester	Particle size (mm)	Parameter Estimation for the Modified Gompertz kinetic model				
		y_m (L/g VS)	R_m (L/gVS/ day)	λ (day)	R	RMSE
A	0.150-0.212	0.2471	3.22E-03	24.779	0.996	0.004359
B	0.212-0.300	0.2056	2.68E-03	24.779	0.996	0.003627
C	0.300-0.600	0.1196	2.55E-03	21.167	0.998	0.002501
D	0.600-1.000	0.0860	2.29E-03	15.430	0.998	0.001796
E	1.000-1.700	0.0788	2.07E-03	18.652	0.997	0.00216

The result of parameter estimation shown in Table 5 revealed that the modified Gompertz equation produced similar correlation coefficients as that of the fractal-like model developed in this study and the model verification are as shown in Fig. 6-10. Further, model evaluation to understand how the fractal-like model performed with respect to its ability to predict cumulative biogas yield was compared with the performance of the modified Gompertz equation. The second-order Akaike Information Criterion (AIC_c) represented by Eq. (19) was utilized for the performance evaluation and this method usually penalizes models with higher number of parameters.

$$AIC_c = n \log \left(\frac{RSS}{n} \right) + 2(p) + \frac{2(p)(p+2)}{(n-p-2)} \quad (19)$$

$$\text{Where, } RSS = \sum_{i=1}^n (Y_{\text{exp}} - Y_{\text{pred}})^2 \quad (20)$$

Where RSS is the residual sum of square represented by Eq. (20), where Y_{exp} and Y_{pred} are experimental and predicted biogas yield; n represents the number of data points; p represents the number of parameter of the models. Usually, the model that gives the lowest AIC_c value is automatically considered the best between two compared models because it is believed to be closer to the unknown truth and would lead to less information loss relative to the alternative model (Momoh and Saroj, 2016). However, further insight into the model comparison can be explained using the following recommendations: When the difference in AIC_c ($\Delta_i = (AIC_i - AIC_{\min})$) between two models is less than 2, no difference is believed to exist between the models, Δ_i between 3 and 7 indicates that the model

with AIC_i has considerable less support while, Δ_i greater than 10 indicates that the model with AIC_i is very unlikely to provide support when compared to the model with AIC_{min} . Where, AIC_i and AIC_{min} represent AIC_c value of alternative model and AIC_c value for model with lowest AIC_c value respectively (Momoh and Saroj 2016).

AIC _c values	Digester Label				
	Dig. A	Dig. B	Dig. C	Dig. D	Dig. E
AIC _c (fractal-like kinetic model)	-347.544	-359.516	-384.16	-405.812	-392.835
AIC _c (Modified-Gompertz kinetic model)	-347.66	-359.634	-383.845	-405.42	-393.407
$(\Delta_i = (AIC_i - AIC_{min}))$	0.116	0.117	0.314	0.391	0.571

Table 6: Models AIC_c and corresponding Δ_i for digesters A-E

The application of the second-order Akaike Information Criterion (AIC_c) revealed that both models are comparable in performance since the difference in AIC_c between the two models was less than 2, ($\Delta_i = (AIC_i - AIC_{min})$) (Table 6). The fractal-like model, however may have the added advantage of not having to assume a hypothetical initial gas volume during model development. Also, the maximum biogas yield rate (R_m) and the lag phase (λ) which are peculiar to the modified Gompertz equation can still be derived by the fractal-like model by taking the first derivative and second derivative respectively of equation Eq. 17. The details of such procedure is a subject for another article. Thus, the fractal-like model developed in this study can be utilized to obtain elaborate biological meaningful parameters far above the modified Gompertz equation.

Fig. 6: Model verification for fractal and Modified Gompertz for Dig. A

Fig. 7: Model verification for fractal and Modified Gompertz for Dig. B

Fig. 8: Model verification for fractal and Modified Gompertz for Dig. C

Fig. 9: Model verification for fractal and Modified Gompertz for Dig. D

Fig. 10: Model verification for fractal and Modified Gompertz for Dig. E

4.5 Implication and Application of Fractal-Like Kinetic Model in Anaerobic Digestion Studies

The utilization of ligno-cellulosic biomass for biogas production is seriously limited due to the inaccessible nature of these biomass and poor understanding of the kinetics of ligno-cellulose hydrolysis (Miron et al., 2000 and Gavala et al., 2000). This study has attempted to highlight the major contributory factors surrounding poor inefficient biotransformation of ligno-cellulosic biomass blaming it squarely on the presence of obstacles or inaccessible regions that make these biomass largely hydrophobic in nature. The initial hydrolysis rate constant was identified as a key regulatory factor to control in order to unravel the limitations surrounding the biotransformation of ligno-cellulosic biomass. The initial hydrolysis rate constant which can be expressed as $\left(X_o k'_o \frac{\Delta S}{\Delta X} \right)$ can be seen to be a function of the initial hydrolytic bacteria population, the specific affinity and the inverse of the bacteria yield coefficient. This parameter which is considered in this study to be the rate limiting component should always be appreciably high for improved biogas production and it can be used to characterize the catalytic efficiency of hydrolytic bacteria from different inoculum source. In anaerobic digestion studies, there has been recent acknowledgement of the role of initial bacteria population. For example, Raposo et al (2012) reviewed the factors affecting the performance of anaerobic batch assays, and observed that, even when experimental conditions of batch assays were synchronized, a certain degree of variability always remains in the result of biochemical methane potential. They attributed these difference to the origin of the inoculum, as it comes with different microbial population, leading to differences in initial activity and substrate adaptation (Wittebolle et al., 2009; Regueiro et al., 2012; Gough et al., 2013). Thus, according to De Vrieze et

al., (2015), the estimation of methane production of agro-allied products based on the chemical composition of the substrate may not be sufficient.

Majority of biogas yield kinetic models available are either empirical or derived from classical theory of the substrate being homogenous without taking into account the fractal or heterogeneous nature of the substrate. As such, they fail to predict at larger particle sizes or higher ligno-cellulose concentration. For example, the modified Gompertz model (Ware and Powers (2017)), modified logistic model (Ware and Powers, 2017), the first-order biogas yield model without lag (Momoh and Nwaogazie, 2011) were derived on the concept of homogenous medium. According to Ware and Powers (2017) the modified Gompertz model and modified logistic model are inappropriate for complex biomass. The novelty of the developed fractal-like model thus, resides in the model uniqueness in considering the contributions of the inoculum (initial micro-organism, X_o) utilized in anaerobic digestion, which has before now, being neglected in other existing models. Also, this model approach provide biological and physiological meaningful parameters that are associated with hydrolysis of ligno-cellulosic (complex) biomass which includes;

- (i) The specific affinity of hydrolytic enzyme on ligno-cellulosic substrate, which measures the adsorption of hydrolytic enzyme on ligno-cellulosic substrate.
- (ii) The initial hydrolytic rates, which measures the initial power of the hydrolytic enzymes on the substrate.
- (iii) The fractal exponent which measures the degree of obstacle present on the substrate.

In addition, this modeling technique may help explain the dynamics involved in the shift in hydrolysis been rate limiting to hydrolysis not been rate limiting during anaerobic digestion of complex biomass especially at very high inoculum to substrate ratio.

5. Conclusions

An insight into the anaerobic digestion kinetics of ligno-cellulosic biomass using equivalent weight of rice husk and cow dung was assessed by utilizing a newly developed fractal like kinetic model. This model showed great capabilities in describing hydrolytic process and predicting cumulative biogas yield. Hydrolysis of ligno-cellulosic biomass was shown to be dependent on the degree of inaccessible regions present in ligno-cellulosic biomass which was indexed by the fractal exponent

(h) and also, on the overall catalytic rate which comprised of the initial hydrolytic rate $\left(\frac{X_o}{Y} k'_o\right)$ and overall affinity constant $(k'_o S_o)$. Smaller particle size fractions were associated with lower fractal

exponents, while larger particle size fractions were associated with higher fractal exponents. In general, the fractal-like model was recommended for use in predicting cumulative biogas yield from ligno-cellulosic biomass undergoing anaerobic digestion in batch reactors because, it has the potential of producing elaborate parameters with biological and physiological meaningful relevance.

Conflict of interest

We declare that there was no conflict of interest associated in the preparation of this article.

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Highlights

- Cumulative biogas yield was inversely proportional to particle size fractions.
- Smaller particle size fractions were associated with the smaller fractal exponents.
- Larger particle size fractions were associated with higher fractal exponents.
- Hydrolytic bacteria showed higher specific affinity for the larger particle size fractions.
- Higher hydrolysis rate and biogas yield were associated with smaller particle size fractions.