Development and Testing of Surface-Based and Water-Based-Diffusion Kinetic Models for Studying Hydrolysis and Biogas Production from Cow Manure

BY

Momoh O.L Yusuf[1], Saroj D.P.[2]

[1]Department of Civil and Environmental Engineering, University of Port Harcourt Choba, P.M.B 5323 Rivers State, Nigeria (yusuf.momoh@uniport.edu.ng)

Phone number: +2348035386779

[2]Department of Civil and Environmental Engineering, University of Surrey, Surrey GU2 7XH, United Kingdom (d.saroj@surrey.ac.uk)

Phone number: +44(0)1483686634

ABSTRACT

The hydrolytic step is usually considered the rate limiting step in the biological conversion of ligno-cellulose material into biofuels. Current optimization approach attempts to understand the mechanism of hydrolysis in order to boost biogas production. In this study, the development and testing of a surface-based and a water-based-diffusion kinetic model for modeling biogas production from cow manure was conducted at ambient conditions using total solid (TS) loading ranging from 8-10% (TS) in batch reactors. Parameter estimation using solver function of the Microsoft Excel Tool Pak revealed that the second order-water based diffusion model was superior in predicting biogas production with a correlation coefficient ranging from 0.9977-0.9995. In addition, the initial surface permeability flux of water (K_{spf}^{0}) into the organic biomass and fragmentation of particles were observed to be independent events elicited by the action C_1 and C_x factors respectively. The initial surface permeability flux of water (K_{spf}^{0}) was observed to increase as cow manure concentration increased from 8-9%TS while, fragmentation constants decreased. Maximum initial surface permeability flux of water (1.78E-05 $m^3/m^2/day$) and maximum initial water uptake rate (k^0_2) (1.86E-05 $m^3/kg/day$) was observed at 9% (TS) with a simultaneous minimization in the rate of fragmentation (0.13/day). For optimal production of biofuels from ligno-cellulose material, appropriate quantity of C_1-factor in cellulase, the degree of crystallinity and particle size may be critical for efficient biomass conversion.

Keywords: C_1 factor, Hydrolysis, Permeability, Biogas Yield, Cow Manure, Batch reactor

1. INTRODUCTION

The utilization of (ligno) cellulose biomass does not only present an attractive option for production of biofuels such as biogas and bioethanol but also provide credible means of promoting sustainable development and addressing issues relating to climate change [1-2]. However, the bioconversion process of cellulose biomass is usually limited because of the crystalline nature of cellulose which hinders enzyme accessibility to the microcrystalline fraction of cellulosic biomass [3]. However, pretreatment options like the thermal, chemical, biological and mechanical processes have been employed to make cellulose more accessible to hydrolytic enzymes, thereby improving the biodegradability [3].

In the anaerobic breakdown of (ligno) cellulose material, hydrolysis step is considered the rate limiting step [4]. Although, detailed knowledge about the mechanism of hydrolysis is still lacking, recent studies by Thygesen et al., [5] using fluorescent-labeled enzymes showed that cellulase was able to access the porous regions of cellulose before any depolymerization could occur. Thus, it has been suggested that enzymatic hydrolysis of cellulose particles could occur by the diffusion of hydrolytic enzymes through pores large enough to accommodate enzyme and then initiate cellulose fragmentation and depolymerization [6-7] or on the external surface, by sequential shaving of cellulose fibrils [8]. In the light of these, two basic models exist in describing the effect of hydrolysis on cellulosic particles and they include, the shrinking particle model (SPM) which assumes that particles do not breakup or fragment but continually reduce by shaving or planning in an onion peeling fashion, and the particle breakup model (PBM) which assumes that particles first breakup or fragment into smaller fragment before depolymerization [9].

Traditionally, the enzyme responsible for degrading cellulose into glucose consist of a suite of enzymes called cellulase that is composed of endo-glucanases, which randomly cleave β-1,4-glycosidic bonds on the cellulose chains away from chain ends; exo-glucanases (cellobiohydrolases), that produce cellobiose by attacking cellulose from chain ends, in which cellobiohydrolase (CBH I) act from the reducing ends while cellobiohydrolase (CBH II) act from the non-reducing ends; and β-glucosidase, that converts cellobiose to glucose [10].

Although, the detailed mechanism for the hydrolysis of cellulose is still unknown, the earliest proposed mechanism developed by Resse et al., [11] known as the C_1-C_x model assumes that, cellulose is initially attacked by a C_1-component (swelling factor) which exposes cellulose to subsequent attack of the C_x-factor (endo and exo-cellulase). The C_1 factor was believed to act in a way that permits increase water uptake by disrupting cellulose and making linkages more accessible to the action of hydrolytic enzymes. Although, this mechanism sound attractive, the swelling factor has eluded researchers for decades, only for the recent discovery of a group of oxidizing enzymes called lytic polysaccharide mono-oxygenase (LPMO) which has been attributed to be a member of the cellulase enzyme capable of disrupting crystalline regions in cellulose [12-13]. On the other hand, another mechanism proposed by Wood and McCrae [14] called the endo-exo model, assumes that the initial attack on bulk cellulose was carried out by the C_x-component (endo-cellulases). This component is responsible for the random attack of amorphous regions of cellulose and is non-processive. Subsequent attack was assumed to be conducted the C_1-component (exo-cellulase) which attack free chain ends and are processive, generating cellobiose as end products [15].

According to Carlos et al., [3] factors known to influence the rate of hydrolysis of lignocellulose biomass in batch reactor include, enzyme related factors such as, mass transfer resistance of the enzyme, the rate of diffusion/adsorption of the enzymes on the surface and rate of cellulase possessive action. In addition, other factors such as substrate complexity and fractals environmental conditions have been proposed to influence the rate of hydrolysis of cellulose [16].

In order to understand the process of hydrolysis, kinetic models have been developed to monitor rate of reducing sugar formation, the rate of acetate production [17] or rate of biogas or methane production [17]. Generally, kinetic models for studying cellulose degradation can be classified into the following; those that depend on bacteria population (enzyme concentration), physical properties of substrate and substrate concentration [18]; those that depend on bacteria population (enzyme concentration) and substrate concentration such as the Michelis Mentens inhibition type equations [3, 9] and those that depend on physical properties of substrate and substrate concentration [9, 19-20].

It is important to note that many of the existing models do not take into cognizance the permeability, diffusion or transfer of water into cellulosic biomass even though hydrolytic enzymes depends strongly on water uptake for their hydrolytic activity. In addition, the role of water can become critical considering the fact that solid organic particles can immediately disintegrate upon coming in contact with water due to dissolution of the soluble component of the organic solid particles [9].

The aim of this study was focused on the development and applications of two kinetic models based on the physical properties of (ligno) cellulose biomass and to validate their efficacy in describing the kinetics of hydrolysis and biogas production. Cow manure was utilized as substrate in this study which, by nature, comprise appreciable portion of (ligno) cellulosic materials and significant micro-flora capable of producing hydrolytic enzymes without the need of external enzyme source during anaerobic digestion.

2. MATHEMATICAL METHOD

The kinetic models developed for studying hydrolysis and biogas production from cow manure were based on the physical properties and concentration of the organic substrate. Similar approach has been utilized for studying hydrolysis of complex biomass by various researchers [9, 19-20]. However, in the model development by authours [19-20], an excess enzyme concentration was assumed to have been utilized to for following hydrolysis of complex biomass. For economic reasons, it may be necessary to consider constant or low enzyme concentration.

2.1 Development of a First Order-Surface Based Kinetic Model

The first order-surface based kinetic model was based on the surface based kinetic expression as proposed by Sanders [19] and modified by Esposito et al., [20-21] who introduced the parameter (a*) to characterize the surface disintegration process. It was assumed that the complex organic substrate particles had similar initial sizes and were spherical in shape.

$$a^{*} = \frac{\sum_{i=1}^{n} A_i}{\sum_{i=1}^{n} M_i} = \frac{nA_i}{nM_i} \quad (1)$$

Where (A) represents the disintegration surface area (m^2) and *M* represents the mass of complex organic substrate (kg) while *n* is the total number of organic particles. The first order-surface based kinetic model developed assumed that disintegration of organic complex is confined only to the surface of the organic biomass resulting in erosion, peeling off or shaving of particles in an onion like fashion [19-20].

In this mechanism, which was assumed to follow the Endo-Exo mechanism as proposed by Wood and McCrae [14], the endo-cellulase (C_X factor) first initiates surface disintegration on the amorphous regions of cellulosic biomass producing free cellulose chains while exo-cellulase (C_1 factor) executed the procession of the free cellulose chains that subsequently led to rapid fiber reduction and ultimate hydrolysis of the cellulosic biomass. These two factors were hypothesized to participate in the mechanism of surface shaving or peeling as depicted in Fig.1. It is important to emphasis that this model best describes the hydrolysis mechanism for smaller size particles or highly amorphous substrates.

The first order hydrolysis of cellulose can be represented by the surface based kinetic model given by,

$$\frac{dC}{dt} = -K_1 C = -K_{sdk}^{o} \bullet a^{*} \bullet C \quad (2)$$

Where *C* is the concentration of complex organic substrate in (kg/m^3); K_{sdk}^{o} represents the initial surface disintegration kinetic constant ($kg/m^2/day$) which is a measure of the initial attack of ligno-cellulosic substrate by endo-cellulase; $\frac{dC}{dt}$ represents the rate of change of complex organic matter ($kg/m^3/day$). However, the expression for a* can be written as Eq. (3) which is a modification to that of Esposito et al., [20-21].

$$a^* = \frac{nA_i}{nM_i} = \frac{4n\pi\left(\frac{d}{2}\right)^2}{\rho n\pi\left(\frac{4}{3}\right)\left(\frac{d}{2}\right)^3} = \frac{6}{\rho d} \quad (3)$$

Where ρ represents the particle density (kg/m^3) of complex organic matter (cow manure) while, d represents the diameter of organic particle. The diameter or particle size was assumed to be reduced following first order kinetics. This assumption was based on reports suggesting that fiber length in cellulose and ligno-cellulosic biomass initially undergoing rapid reduction before any production of reducing sugars was observed during enzymatic hydrolysis ligno-cellulosic biomass [5, 6].

Hence, Eq. (3) could be re-written as,

$$\frac{dC}{dt} = -K_{sdk^o}\left(\frac{6 \bullet C}{\rho d_o \exp(-kt)}\right) \quad (4)$$

Upon integration, Eq. (4) yields Eq. (5)

$$In\left(\frac{C_t}{C_o}\right) = -K_{sdk^o}\left(\frac{6}{\rho d_o k}\right)\left(\exp(kt) - 1\right) \quad (5)$$

Where (k) represent the first order particle size reduction constant (day^{-1}) induced by the C_1 factor leading to rapid disintegration of ligno-cellulose micro-fibers; d_o represents the initial particle size or diameter while (t) represent time in days. However, from work of Linke [22] a correlation can be establish between substrate concentration and biogas yield during anaerobic degradation, such that,

$$\left(\frac{y_m - y_t}{y_m}\right) = \left(\frac{C_t}{C_o}\right) \quad (6)$$

Where y_m and y_t represents the maximum biogas yield and biogas yield at time (t) respectively, expressed as m^3/kg Volatile Solids (VS). Such that equation (6) can be re-written as,

$$y_t = y_m - y_m \exp\left(\frac{-6K_{sdk^o}}{\rho d_o k}\left(\exp(kt) - 1\right)\right) \quad (7)$$

It is important to note that the product of initial surface disintegration kinetics constant (K_{sdk}^{o} (kg/m^2/day)) and the surface disintegration coefficient (a* (m^2/kg)) equals the time dependent first order hydrolysis rate constant (K_1 (day^{-1})) such that,

$$K_1 = K_{sdk^o} \bullet a^* = \frac{6 \bullet K_{sdk^0}}{\rho d} = \frac{6 \bullet K_{sdk^0}}{\rho d_o \exp(-kt)} = 6 \bullet k_1^{\,0} \exp(kt) \quad (8)$$

Where K_1 (day^{-1}); represents the overall first order hydrolysis rate. The initial surface disintegration kinetic constant ($K_{sdk}^{\,o}$) is an important kinetic parameter that measures the initial surface area attack on the cellulosic particles by the C_x factor. This attack leads to the exposure of the complex biomass for further attack by the C_1 factor resulting in rapid particle size or fiber reduction that can be described using the first order exponential function with a first order degradation constant (k).

However, if the lag phase prior to commencement of particle size or fiber reduction is considered one obtains,

$$K_1 = 6 \bullet k_1^{\,0} \exp(k(t - \lambda)) \quad (9)$$

In addition, it can be shown from Eq. (8) that,

$$K_{sdk^o} = \rho d_o k_1^{\,0} \quad (10)$$

Thus, Eq. (7) can be re-written as Eq. (11). It can be observed that the $K_{sdk}^{\,0}$ is directly proportional to the density and particle size of the substrate, while k^0_1 is inversely proportional to the density and particle size. Because it is difficult to obtain a consistent particle size in bulk substrate solution, it will be more convenient to use the reduced form of Eq. (11).

$$y_t = y_m - y_m \exp\left(\frac{-6 k_1^{\,o}}{k}\left(\exp(kt) - 1\right)\right) \quad (11)$$

Where $k_1^{\,o}$ (day^{-1}) represent the initial first order constant for the surface attack of the ligno-cellulosic biomass by the C_x factor. However, if the lag phase for commencement of fiber disintegration or particle size reduction (λ (days)) is considered in the model development, then Eq. (11) can be re-written as,

$$y_t = y_m - y_m \exp\left(\frac{-6 k_1^{\,o}}{k}\left(\exp(k(t - \lambda)) - 1\right)\right) \quad (12)$$

2.2 Development of a Second Order-Surface Water Diffusion model

The second order-surface diffusion model developed in this study assumed that water diffusion into organic biomass and fragmentation of organic complex biomass were critical for hydrolysis. In this mechanism, which was assumed to follow the C_1-C_x mechanism as proposed by Resse et al. [11], it was hypothesized that the binding of a C_1 factor leads to disruption of crystalline cellulose that subsequently allow water to penetrate through the surface leading to the swelling of micro fibrils forming hydrated cellulose. Further attacked by the C_x factor (endo and/or exo-cellulase),) initiates the rapid fragmentation of organic ligno-cellulose biomass into smaller particle. The C_1 factor in conjunction with the hydrolytic enzymes (C_x factor) was assumed to promote surface disruption and fiber or particle size fragmentation of the complex organic biomass respectively before ultimate hydrolysis. In addition, it is important to emphasize that this model concept best describes the hydrolysis mechanism appropriate for larger size ligno-cellulosic particles or highly crystalline substrate. This mechanism is depicted as shown in Fig. 1.

This hypothesis was appropriately captured by a second order hydrolysis of cellulose can be written as a second order-surface water diffusion model given by Eq. (13),

$$\frac{dC}{dt} = -K_2 C^2 = -K_{spf^0} \bullet a^{**} \bullet C^2 \quad (13)$$

Where K_2 is the overall second order water uptake rate (m^3/kg/day); $K_{spf}^{\ 0}$ is the initial surface permeability flux (m^3/m^2/day) of water into the ligno-cellulose after attack by the C_1 factor; and a^{**} the fragmentation coefficient which can be represented by Equation (14)

$$a^{**} = \frac{nA_i}{nM_i} = \frac{4n\pi\left(\frac{d}{2}\right)^2}{\rho n\pi\left(\frac{4}{3}\right)\left(\frac{d}{2}\right)^3} = \frac{6}{\rho d} = \frac{6}{\varphi_v} = \frac{6}{\varphi_{vo}\ \exp(-k_v t)} \quad (14)$$

Where φ_v represents the fragmentation coefficient (kg/m^2) which was assumed to time dependent following a first order fragmentation represented by Eq. (15),

$$\varphi_{vo} \exp(-k_v t) \quad (15)$$

The term φ_v implies that the mass, volume and diameter of the organic particle are changing with time due to the fragmentation process. Hence, substituting Eq. (14) into Eq. (13) one obtains,

$$\frac{dC}{dt} = -K_{spf^0}\left(\frac{6 \bullet C^2}{\varphi_{vo}\ \exp(-k_v\, t)}\right) \tag{16}$$

Upon integration equation (10) one obtains,

$$\frac{1}{C_t} = \frac{6\,K_{spf^0}}{k_v\ \varphi_{vo}}\left(\exp(k_v t)\ -1\right) + \frac{1}{C_o} \tag{17}$$

In terms of biogas yield one obtains Eq. (18)

$$\frac{y_m}{(y_m - y_t)C_o} = \frac{6\,C_o\ K_{spf^0}\ (\exp(k_v t) - 1) + \ k_v\ \varphi_{vo}}{C_o\ k_v\ \varphi_{vo}} \tag{18}$$

This can be re-written as,

$$y_t = y_m - \left(\frac{y_m k_v\ \varphi_{vo}}{6\,C_o K_{spf^0}\ (\exp(k_v t) - 1) + \ k_v\ \varphi_{vo}}\right) \tag{19}$$

However, if the lag phase (λ) is considered in the model development, then Eq. (19) can be re-written as,

$$y_t = y_m - \left(\frac{y_m k_v\ \varphi_{vo}}{6\,C_o K_{spf^0}\ (\exp(k_v (t-\lambda)) - 1) + \ k_v\ \varphi_{vo}}\right) \tag{20}$$

It is important to note that the product of initial surface permeability flux constant ($K_{spf}^{\ o}$ ($m^3/m^2/day$) and the fragmentation coefficient (a** (m^2/kg)) equals the time dependent overall second order water uptake rate constant (K_2 ($m^3/kg/day$) such that,

$$K_2 = K_{spf} \bullet a^{**} = \frac{6 \bullet K_{spf^0}}{\rho\, d} = \frac{6 \bullet K_{spf^0}}{\varphi_v} = \frac{6 \bullet K_{spf^0}}{\varphi_{vo}\ \exp(-k_v t)} = 6 \bullet k_2{}^{o}\ \exp(k_v t) \tag{21}$$

In addition, it can be shown from Eq. (21) that,

$$K_{spf^0} = \varphi_{vo}\, k_2{}^{0}$$

Where k_2^0 represents the initial water uptake rate constant (m^3 /kg /day). It can be observed that the K_{spf}^0 is directly proportional to the density and particle size of the substrate, while k^0_2 is inversely proportional to the density and particle size. However, k^0_2 may be a better measure of water uptake because it is often difficult to obtain a consistent particle size in bulk substrate solution.

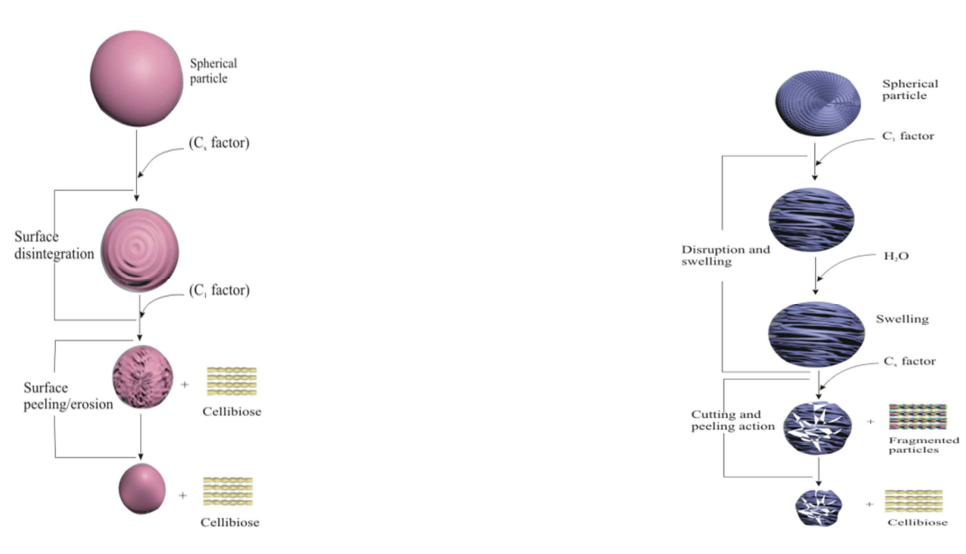
Hence, Eq. (19) can be reduced to Eq. (22),

$$y_t = y_m - \left(\frac{y_m k_v}{6 C_o k_2^{\,o} \left(\exp(k_v t) - 1\right) + k_v} \right) \tag{22}$$

Where k_2^0 represents the initial water uptake rate constant (m^3 /kg /day) by ligno-cellulose biomass after attack by the C_1 factor. However, if the lag phase (λ) is considered in the model development, then Eq. (20) can be re-written as,

$$y_t = y_m - \left(\frac{y_m k_v}{6 C_o k_2^{\,o} \left(\exp(k_v (t - \lambda)) - 1\right) + k_v} \right) \tag{23}$$

The second order hydrolysis rate model developed in this study assumes that the overall water uptake rate (K_2) is inversely dependent on the density (nature of the substrate) and initial diameter of organic particles and directly dependent on the initial surface permeability flux (K_{spf}^0) of water into organic particles. Alternatively, the second order overall water uptake rate (K_2) can be said to be directly dependent on the initial water uptake rate (k_2^o) into ligno-cellulose biomass and fragmentation constant (k_v) induced by the C_x-factor. This derivations support the finding of Hills and Nakano [23] and Sharma et al., [24] who observed that a linear relationship existed between the gas production rate and the inverse of the particle diameter. It therefore implies that the morphology of biomass with respect to shape, density, and all the factors related to density such as porosity, degree of polymerization, the degree of crystallinity and amorphous nature of cellulosic material, may contribute in affecting the rate of hydrolysis of ligno-cellulose biomass.



(a) **(b)**

Fig.1: (a) The first order–surface based kinetic model (endo-exo model) and (b) second order-water diffusion model (C_1-C_x mechanism)

3. EXPERIMENTAL METHOD

Cow manure used in this research was acquired from abattoir situated at Choba community of Rivers State, Nigeria. A mass of 500g cow manure was sun dried and crushed using mortar and pestle while weighing of the crushed cow manure was conducted with a weighing balance (Mettler, model PN163) manufactured in Switzerland with specification ranging from 0.1mg and 160g. The crushed cow manure was loaded into Buchner (batch) flasks labeled A, B, C and D consisting total solid concentration of 8, 8.5, 9 and 10% respectively. The digesters were set-up as described by Momoh and Nwaogazie [25] and set-ups were conducted in duplicates which were within range of 4-12% total solids as suggested by Tchobanglous et al., [26] for low solid loading anaerobic digestion. Volatile solids content of the cow manure was determined before the digestion process commenced according to APHA [27] using a muffle furnace (Carbolite model LMF 4, UK). Similarly, the carbon to nitrogen ratio of the cow manure was determined in accordance with APHA [27].

The crushed cow manure was subsequently loaded into Buchner flasks and corked to exclude air. The digesters were allowed to run in the absence of oxygen for a period 42 days and agitated twice daily at an average ambient temperature of 28 ± 3^0C. The Water displacement method was used to measure biogas production, while the displaced water was saturated brine solution which

prevented the dissolution of carbondioxide in the water. The compositions of digesters A-D are as shown below:

Digester A: Comprised 8% (TS) concentration of cow manure which translated to 61.22g VS/L.

Digester B:Comprised 8.5% (TS) concentration of cow manure which translated to 65.40g VS/L.

Digester C: Comprised 9% (TS) concentration of cow manure which translated to 69.63g VS/L.

Digester D: Comprised 10% (TS) concentration of cow manure which translated to 78.22g VS/L.

4. RESULTS AND DISCUSSIONS

In this study, cow manure which is a pre-treated lignocellulose biomass was utilized as substrate with total solids (TS) ranging from 8 to 10% total solids. Volatile solids were determined to be 70.40% of total solids while carbon: nitrogen ratio was 25:1. The daily biogas production from cow manure revealed that biogas production lasted for 42 days and a period of lag phase usually preceded biogas production in each digester. Biogas productions from digester set-up A-D were used to follow the process of hydrolysis of ligno-cellulose (cow manure) biomass. Model testing involved the utilization of the average cumulative daily biogas yield as the input variable data, and parameter estimation was conducted using solver function of the Microsoft Excel Tool Pak. The results of model calibration for the first order-surface based and second order water diffusion models are presented in Table 1-2. It is important to note that Eq. (9) was used for calibrating the first order- surface based kinetics while Eq. (20) and (21) were used for calibrating the second order- water diffusion model.

It was observed that both the first and second order models provided very high correlation coefficients (r) ranging from 0.9974-0.9994 for the first order-surface based kinetic model and from 0.9987-0.9995 for the second order-water diffusion kinetic model. This implied that both models have the likelihood to be employed for studying the kinetics of hydrolysis and biogas production from cow manure. However, by considering the root mean square errors (RMSE), the second order-water diffusion kinetic model was observed to adequately provide the lower root mean square error (RMSE) for all the data set studied.

However, for proper model selection the second-order Akaike Information Criterion (AIC_c) represented by Eq. (24) was also utilized. This method of model selection usually penalizes models with higher number of parameters. The best model usual has the lowest AIC_c value [28].

$$AIC_c = n\log\left(\frac{RSS}{n}\right) + 2(p) + \frac{2(p)(p+2)}{(n-p-2)} \quad (24)$$

Where, $$RSS = \sum_{i=1}^{n} \left(Y_{\exp} - Y_{pred}\right)^2 \quad (25)$$

Where RSS is the residual sum of square represented by Eq. (25), 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. Where the difference in AIC_c $\left(\Delta_i = \left(AIC_i - AIC_{\min}\right)\right)$ between two models is less the 2, no difference is believed to exist between the models. However, 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 for predictive purposes when compared with the model with AIC_{min} [28] 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. Table 3 shows the evaluated AIC_c and corresponding Δ_i for digesters A-D.

Hence, the second order-water diffusion kinetic model was considered superior in validating biogas production from anaerobic digestion of cow manure, because it produced the lower AIC_c values and also, the difference in AIC_c values obtained for both models was more than 2 in all the digesters under study. This finding implies that larger-sized crystalline particles may have constituted greater fraction of the biomass that participated in the hydrolysis process. However, this does not imply that the first-order surface based kinetic model is less superior in validating biogas production from anaerobic digestion of cow manure. The first-order surface based kinetic model may become significant if smaller-sized amorphous particles constituted greater fraction of the biomass participating in the reaction. Thus, model verification and subsequent discussions were limited to the second order-water diffusion model.

Table 3: Parameter Estimation for the first order-Surface based kinetic model

Digester	Volatile solids Conc (kg/m^3)		Parameter Estimation for the first order-Surface based kinetic model					
			y_m $m^3/kgVS$	k^o_1 (day^{-1})	k (day^{-1})	λ (days)	r	RMSE
A	61.22	8%	0.0599	1.19E-03	0.137	3.42	0.9992	7.88E-04
B	65.40	8.5%	0.0605	1.67E-03	0.111	2.56	0.9987	1.13E-03
C	69.63	9%	0.0673	1.9E-03	0.091	3.39	0.9974	1.68E-03
D	78.22	10%	0.0688	1.36E-03	0.107	3.16	0.9994	9.74E-04

Table 2: Parameter Estimation for the second order- Water diffusion kinetic model

Digester	Volatile solids Conc (kg/m^3)		Parameter Estimation for the second order- Water diffusion kinetic model							
			y_m $m^3/kgVS$	K_{spf}^0 ($m^3/m^2/day$)	$\varphi_o(kg/m^2)$	k^o_2 ($m^3/kg/day$)	k_v (day^{-1})	λ (days)	r	RMSE
A	61.22	8%	0.063	1.04E-05	1.73	6.04E-06	0.201	0.47	0.9995	6.84E-04
B	65.40	8.5%	0.064	1.36E-05	1.14	1.2E-05	0.167	0.84	0.9992	9.08E-04
C	69.63	9%	0.074	1.78E-05	0.95	1.86E-05	0.130	2.96	0.9977	1.53E-03
D	78.22	10%	0.076	1.59E-05	1.45	1.1E-05	0.145	2.43	0.9995	6.76E-04

Table 3: Models AIC_c and corresponding Δ_i for digesters A-D

AIC_c values	Digester Label			
	Dig. A	Dig. B	Dig. C	Dig. D
AIC_c (first –order Surface based kinetics)	-251.34	-238.18	-223.69	-243.60
AIC_c (second–order water diffusion kinetics)	-256.50	-246.16	-227.69	-256.92
$(\Delta_i = (AIC_i - AIC_{\min}))$	5.16	7.98	3.38	13.32

4.1. Effect of Solid Loading on Kinetic Constants

By considering the second order-water diffusion model, it was observed that as solid loading increased from 8 to 9%, the initial surface permeability flux (K_{spf}^{0}) of water into the ligno-cellulose biomass linearly increased while the fragmentation rate constant (k_v) linearly decreased (Fig.2). Also, for this same range of organic loading, the initial water uptake (k_2^o) was observed to increase linearly (Fig.3). It was hypothesized that increase in the initial water uptake rate (k_2^o) could be attributed to the disruption of the complex biomass by binding of the C_1 factor which exhibited peak saturation at 9% total solids (Fig 2-3). The increase in the activity of C_1 factor seemed to correspond with a decrease in activity or binding of endo and/or exo-cellulase (C_X factor) which was reflected in the reduced fragmentation constants obtained as solid concentration increased (Fig. 2).

Early evidence of a water uptake factor was indirectly captured in the works of Felby et al., [29] who showed that by observing the T_2 distribution pattern using NMR technique, water uptake was highest when celluclast (a commercial enzyme from Trichoderma Ressei, which already contain the C_1 factor) was utilized to digest filter paper as compared to the action of endo-cellulase alone or exo-cellulase (Cellobiohydrolase) alone (devoid of the C_1 factor). Hence, water uptake factors (C_1 factor) in conjunction with the fragmentation factors endo and/or exo-cellulase (C_X factor) may independently play essential roles in the hydrolysis of ligno-cellulose biomass (cow manure).

Also, from Fig. 2-3, the drop in initial surface permeability flux (K_{spf}^{0}) of water and the initial water uptake beyond 9% total solids after a peak value, suggests that the C_1 factor may be saturative in nature and possibly a discrete entity from the C_X factor (cellulase enzymes) and that, they may be produced in lower quantity than other cellulase enzymes. This suggestion was reached because fragmentation process was observed to increase slightly at 10% TS with a corresponding reduction in the initial water uptake rate after saturation of C_1 factor. The slight increase in the fragmentation constant may be attributed to the reduced action of C_1 factor beyond 9% TS. This effect is reflected in a drop in the initial surface permeability flux or initial water uptake beyond 9% total solids concentration. This finding implies that, although the C_1 factor may help expose ligno-cellulose surface to further attack by the C_x factor (endo and/or

exo-cellulase) by allowing water into the ligno-cellulose biomass, their activity may actually slow down the activity of C_x factor (endo and/or exo-cellulase). Possible explanation for this observation is that the C_1 factor may either have the potential to out-compete the endo and/or exo-cellulase for binding sites or that the process of water passage into biomass may slow down the activity of the C_x factors (endo and/or exo-cellulase). However, because the C_1 factor has been suggested to attack crystalline region of cellulosic material rather than the amorphous regions (Hu et al., 2014), it was most likely that C_1 factor slow down exo-cellulase binding on crystalline region of ligno-cellulose biomass. This suspicion was recently confirmed from works of Eibinger et al., [30] who discovered that an oxygenase enzyme called lytic polysaccharide mono-oxygenase (LPMO) which actually possess crystalline surface disruptive abilities may also potentially compete with exo-cellulase (CBH I).

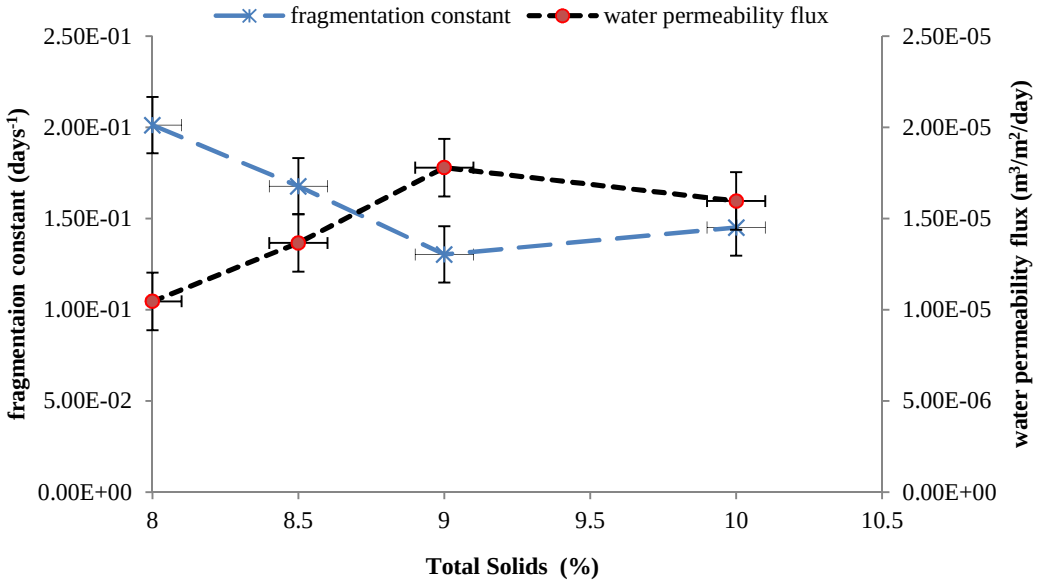
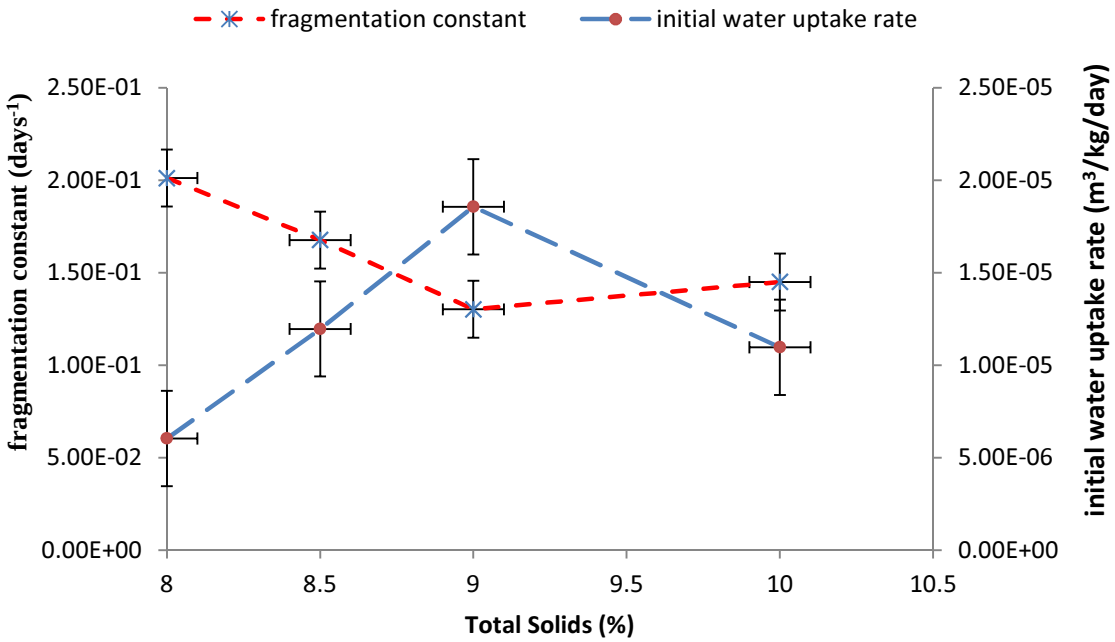


Fig.2: Effect of totals concentration on fragmentation constants and water permeability flux



423

424 **Fig.3: Effect of totals concentration on fragmentation constants and initial water uptake**
425 **rate**

426 Lytic polysaccharide mono-oxygenases (LPMO) are a group of oxidative component of cellulase
427 mixture that act cooperatively with endo, exo-cellulase and beta-glucosidase to hydrolyze
428 crystalline cellulose which are now considered to be the C_1 factor proposed by Reese in 1950
429 [12], [30]. However, recent study have revealed the possibility of competition between LPMO
430 and exo-cellulase (CBH I) (used alone) and a clear lack of synergy during hydrolysis of a mixed-
431 amorphous-crystalline cellulosic substrate. On the contrary, for highly crystalline substrate like
432 Avicel and Nano-crystalline cellulose, synergy was observed between LPMO and exo-cellulase
433 (CBH I) [30], thus implying that the nature of synergy was dependent on the relative amount of
434 accessible crystalline to amorphous cellulose within a substrate [12].
435
436 It is interesting to note that, in addition to the above factors highlighted by these researchers, this
437 study has also establish the fact that, the concentration of bound C_1 factor per unit area of
438 crystalline cellulose material (which was followed by the initial surface permeability flux of
439 water (K_{spf}^{0}) into the biomass in this study) has major role to play in regulating hydrolysis in
440 general. Low activity/binding of C_1 factor (which was followed herein as the initial surface

permeability flux of water into biomass) was shown to improve fragmentation kinetics while, high activity/binding of C_1 factor or high initial surface permeability flux of water into biomass reduced fragmentation kinetics (Fig. 2). Hence, the degree (concentration) of activity/binding of the C_1 factor and the relative amount of accessible crystalline to amorphous cellulose within a substrate may all contribute in determining degree of synergy and hydrolysis of crystalline cellulosic biomass. This relationship is captured in Table 4 where it is proposed that a high concentration of C_1 factor in cellulase usage against a low crystalline or low concentration of crystalline substrate may not be too beneficial due to the low fragmentation and prolong time of hydrolysis that may ensue. Also, the competitive effect of C_1 factor may help explain why amorphous cellulose is rapidly consumed faster than crystalline cellulose.

Table 4: Degree of Synergy for Hydrolysis of Crystalline Cellulose with Cellulase Suite

Degree of activity/binding of C_1 factor per unit area of biomass	**Degree of crystallinity**			
	Low	**Moderate**	**High**	**Very high**
Low	+	++	+++	++++
Moderate	--	+	++	+++
High	---	--	+	++
Very high	----	---	--	+

+ represents positive synergy - represents negative synergy

In general, a mathematical relationship may be used to describe the overall water uptake rate observed during cellulose hydrolysis that is, the overall water uptake process is dependent on the initial water uptake rate (C_1 factor), the fragmentation factor (endo and/or exo-cellulase activity) and also the shape of the organic particles as (shape factor) shown in the relationship represented by Eq. (21),

$$K_2 = 6\downarrow_{shape\ factor} . k_2^{\ o} \downarrow_{C_1\ factor} \exp(_{fragmentation\ factor}\downarrow k_v t)$$

The overall water uptake coefficient (K_2) seems to be dependent on the shape factor, which is 6 for spherical particles and 4 for cylindrical particles, [17], the initial water uptake rate by the

organic biomass (k_2^o) induced by the C_1 factor and also the fragmentation constant by C_x factor (endo and/or exo-cellulase). It therefore implies that the morphology of biomass with respect to the shape, the density the degree of crystallinity and amorphous nature of cellulosic material (with highly crystalline cellulose biomass showing high reactivity to the C_1 factor than amorphous cellulose biomass [12]) may contribute to enhance or resist the entire hydrolysis process

In essence, experimental studies may be affected by the source of cellulase enzyme, the particle size of cellulose, the degree and concentration of crystallinity (with highly crystalline cellulose or high concentration of crystalline cellulose showing high affinity for the C_1 factor than the amorphous cellulose) thus, implying that crystalline cellulose will show slower fragmentation kinetics and lesser positive synergism (slower rate of hydrolysis or longer hydrolysis time) because of reduced fragmentation action, following possible competitive relationship between C_1 factor and exo-cellulase (CBH I) as compared to the hydrolysis of amorphous cellulose because amorphous cellulose has low affinity for the C_1 factor. Thus, amorphous cellulose would tend be hydrolyzed much faster than crystalline or partially crystalline cellulose when digested with cellulase containing the C_1 factor. These observations have been reported in works of various authours such as Gao et al., [31] and Samejima et al., [32]. Hence, the binding and disruption of crystalline surface of cellulosic biomass by C_1 factor may be the regulatory step in the hydrolysis of crystalline cellulose or ligno-cellulose biomass.

4.2 Application of water diffusion model for studying the Hydrolysis rate and biogas yield from cow manure

In this study, it was proposed that the process of hydrolysis of cow manure may follow the second order water diffusion kinetic model that depends strongly on the uptake of water into the organic biomass after which the products of hydrolysis are converted into biogas during the anaerobic process. Fig, 4-5 shows the progress of water uptake during the anaerobic degradation of cow manure. In general, as solid loading increased from 8 to 10% TS, it was observed that the time dependent overall water uptake rate (K_2) increased with time. However, the overall water uptake rate (K_2) at the end of the digestion period of 42 days was observed to drop as solid loading increased from 8 to 10% TS. It can be observed that solid loading at 8 and 8.5% TS

showed distinct pattern in their overall water uptake rate while solid loading at 9 and 10% showing almost similar patterns in their overall water uptake rate. Hence, by following the time dependent overall water uptake rate (K_2), the effectiveness of the biogas conversion process can be access. Higher trends in the overall water uptake rate (K_2) tend to depict lower biogas yield while lower trends in the overall water uptake rate (K_2) tend to depict higher biogas yield. Hence, from Fig 4-5, the total solid concentration corresponding to optimum biological activity could be set at 9% TS.

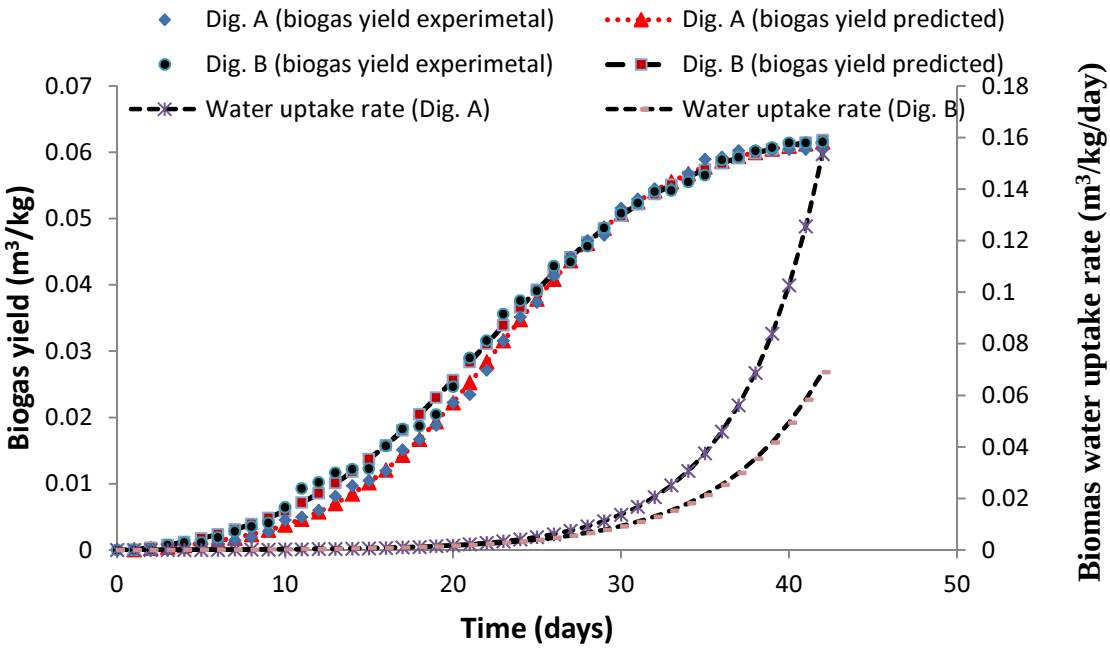


Fig 4: Validation of biogas yield and simulation water uptake rate (Dig. A-B)

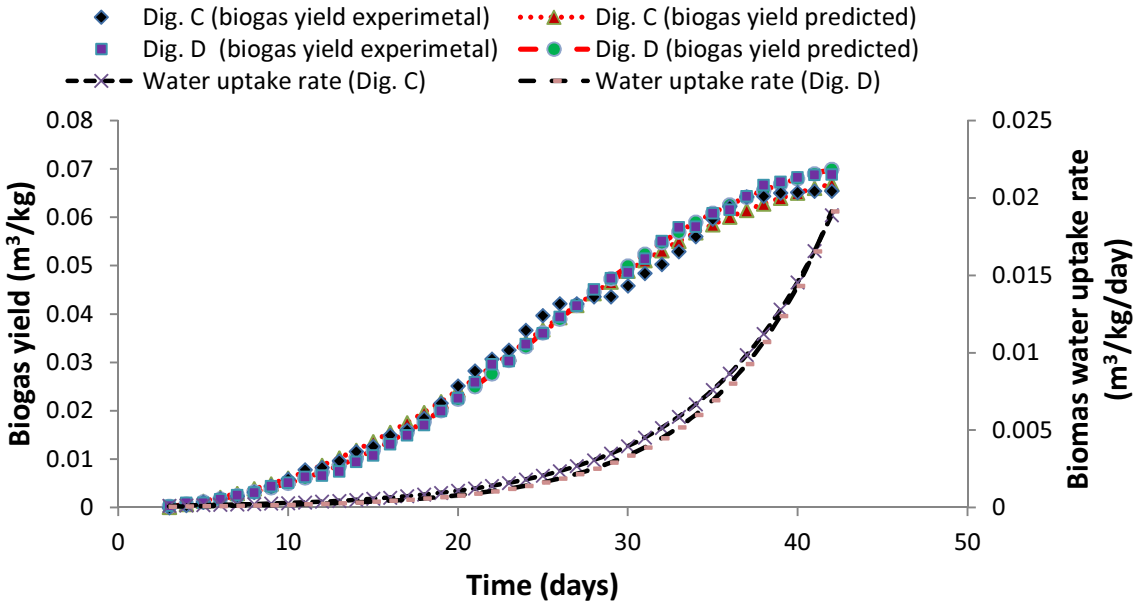


Fig. 5: Validation of biogas yield and simulation water uptake rate (Dig. C-D)

Furthermore, the model verification of biogas production using the second order-water diffusion model (Fig.4-5) suggests that the model can appropriately predict biogas yield by taking into cognizance the lag phase period. The lag phase period estimated (shown in Table 2) seemed to relate more to the lag periods prior to commencement of fragmentation process rather than the lag periods prior associated with biogas production. The flexibility of this model is depicted in its ability to predict biogas yield with lower AIC_c values.

4.3. Fragmentation and Hydrolysis process during anaerobic degradation of Cow Manure

It has been established in this study that, fragmentation is propelled by the action of hydrolytic enzymes whose activity generally slows down with increasing substrate loading. However, the process of fragmentation can be followed by the plotting the fractional fragmentation remaining versus time using the relationship,

$$\alpha = \frac{\varphi_v}{\varphi_{ov}} = \exp\left(-k_v(t-\lambda)\right) \tag{24}$$

The plot of the fractional fragmentation remaining versus time (Fig. 6) revealed that the process of fragmentation of bulk organic biomass (cow manure) lasted only for a short period of time that span over 10 days. This process of fragmentation involved disintegration of bulk organic biomass into smaller particles during the early period of enzymatic saccharification. Similar findings have been reported by Peters et al., [33] who observed a rapid reduction in particle size during hydrolysis of microcrystalline cellulose in the early period of hydrolysis. Also, Arantes et al., [34] observed rapid reduction in fiber length prior to slow down of hydrolysis. They concluded that fragmentation is a rapid but not a continuous process required to maintain effective hydrolysis for large particle sized biomass. The discontinuity of fragmentation process after a short period of about 10days as observed in this study, confirms the report of Peters et al., [33] and Arantes et al., [34].

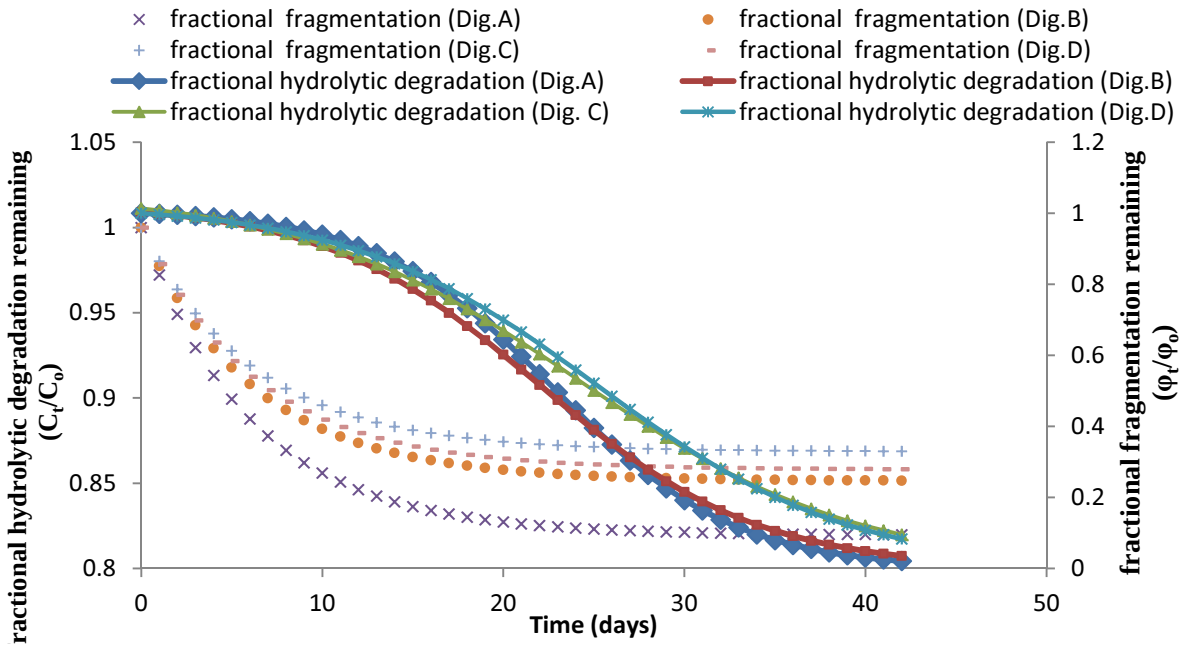


Fig. 6: Simulating ligno-cellulose (cow manure) hydrolysis and fragmentation process with time

Furthermore, the second order-water diffusion model developed in this study can be used to simulate the hydrolysis of cow manure. By applying Eq. (25), the fraction of cow manure volatile solids concentration remaining (C_t/C_o) during the anaerobic digestion could be simulated.

$$\frac{C_t}{C_o} = \left(C_o \left(\frac{6 k_2{}^o}{k_v} \left(\exp(k_v (t - \lambda)) - 1 \right) + \frac{1}{C_o} \right) \right)^{-1} \quad (25)$$

From Fig. 6, it is shown that changes in cow manure concentration were relatively constant during the first 10days of anaerobic digestion. However, gradual hydrolysis enabled decrease in cow manure concentration to become evident after 10days in all the digesters simulation study. These findings are consistent with report of Chauve et al., [35] and Arantes et al., [34] who observed a rapid fragmentation followed by a slow erosion of cellulose fiber during hydrolysis process.

5. CONCLUSIONS

The ability of the second order water-based diffusion kinetic model to effectively describe biogas production and simulate hydrolysis behavior implies that the crystallinity of the substrate played a significant role in describing the hydrolysis process. In addition, a water swelling factor identified as C_1-factor was established to operate in association with fragmentation of ligno-cellulase substrate. The overall hydrolysis rate coefficient (K_2) was observed to be a time dependent exponential function that depends on the morphology or shape of substrate particles, the initial water uptake by the cellulose biomass and the fragmentation constant. In addition, the C_1 factor was suggested to exhibit competitive behavior with the hydrolytic enzymes, such that, large scale utilization of ligno–cellulose materials for improved biogas or bioethanol product will largely be dependent on an appropriate mix ratio between C_1 factor, cellulase and the crystalline cellulose or ligno-cellulose substrate. Also, optimization of biological processes for efficient transformation of ligno-cellulose in biofuels can be achieved via this modeling technique. In addition, the models developed in this study may open vista of opportunities into elucidating the detailed mechanism of the very complex process of ligno-cellulose hydrolysis.

CONFLICT OF INTREST

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

ACKNOWLEDGMENT

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