Biodegradability and kinetic studies on biomethane production from okra (*Abelmoschus esculentus*) waste

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Emerging from the energy crisis of 2008 in South Africa, climate change concerns and the global desire to reduce high ozone-depleting emissions, renewable energy sources like biogas are gaining wide acceptance in most localities for heating and electricity. The paucity of feedstock varieties is a major challenge plaguing the sustainability of this sector. Biomethane potential, biodegradability and degradation kinetics of organic substrates are essential for assessing the suitability of feedstocks for methane generation and the overall performance of the anaerobic digestion process in biogas plants. Waste from the vegetable okra (*Abelmoschus esculentus*) is a novel substrate; its biodegradability and degradation dynamics in biomethane production are largely unstudied, and were therefore the aims of this research. The substrate was digested for 25 days at the mesophilic condition and the biomethane potential data were recorded. Measured data of methane yield and the elemental composition of the substrate were used to fit five models (modified Gompertz, Stannard, transference function, logistic and first-order models) to predict degradation parameters and determine biodegradability of the substrate, respectively. Low lag phase (0.143 d), positive kinetic constant (0.2994/d) and the model fitness indicator (<10) showed that transference and first-order kinetic models predicted the methane yield better than did other growth functions. The experimental methane yield was 270.98 mL/gVS, theoretical methane yields were 444.48 mL/gVS and 342.06 mL/gVS and model simulation ranged from 267.5 mL/gVS to 270.89 mL/gVS. With a prediction difference of 0.03–1.28%, all growth functions acceptably predicted the kinetics of *A. esculentus* waste. The findings of this study offer information on this novel substrate important for its use in large-scale biogas production.

Significance:

- Growing interest in biogas technology as an alternative energy source for both South African rural dwellers and industries, has mounted enormous pressure on known feedstocks, and instigated the search for novel substrates.
- Our study shows that okra waste is a viable feedstock for biogas production.
- The suitability of the first-order kinetic model over other models in predicting okra waste degradation was highlighted.

Introduction

Global concerns regarding the depletion rate of fossil fuel sources, their adverse impacts on the environment and the need to reduce the emission of greenhouse gases, have necessitated overwhelming interest in unconventional energy sources from biomasses and wastes.[1-3] Biogas technology is a renewable energy type, which combines sustainable waste management and efficient biofuel production.[4] This waste-to-energy (biogas) process is an established technology, but it has been underexploited in most developing climes like South Africa. According to the South African Biogas Industry Association[5] and Damm and Triebel[6], more than 2.328 million households (about 25% of all families in South Africa) use local fossil fuel sources like charcoal and firewood to meet their energy demands. The high cost and unavailability of electricity in most informal and rural settlements has increased both the demand for and development of biogas technologies.[7]

Anaerobic digestion is a clean energy recovery process of biogas production through the biological degradation of organic wastes in the absence of oxygen for the generation of methane.[8] This biomass degradation by microbes reduces the volume of waste and involves four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis.[2,8,9]

According to Bharathiraja et al.[9], the low cost, availability and novelty of feedstocks are the incentives needed for more investment in biogas production. This has necessitated the aggressive search for future energy crops with potential for ensuring feedstock security, optimisation of existing biomass feedstocks as well as the technological enhancement of feedstock digestion processes.[9] Various efforts towards discovering novel biomass for biogas have been made. Adiga et al.[10], Patil et al.[11], Bai-Hang et al.[12] and Visva Bharati et al.[13] studied the enhancement of water hyacinth for biogas production. Anongnart et al.[14], Rodriguez et al.[15] and Kroger and Muller-Langer[16] noted that both micro- and macro-algae is a viable substrate for biogas production. Housagul et al.[17] and Aguilar-Aguilar et al.[18] investigated the use of glycerol from biodiesel industries, singly and in combination, for biogas production, while Li et al.[19] attempted to co-digest 33–53% spent cooking oil with food waste. Other novel substrates investigated include meadow grasses[20] and vegetables[21].

In countries such as China, over 200 million tons of about 700 million tons of vegetables produced annually ends up as residues and waste.[22] Okra (*Abelmoschus esculentus*) waste is a vegetable waste-type, which is largely novel and has thus far been unexploited for biogas production. Okra is mainly grown in Africa and India (96% of worldwide production[23]). Okra waste – like that of other vegetables and fruits – accounts for 40–50% of the 48.4%

total food waste globally, a significant volume across its processing value chain.[23] Duman et al.[24] stated that Turkey produces over 36 000 tons of okra per year and that the utilisation of okra waste has been studied as part of Turkey's development plan and vision. Okra and its stems are high in crude fibre, protein and fat; dired okra has about 25% crude fibre and 18% protein.[25] According to Alam and Khan[26], the entire crop waste contains 67.5% a-cellulose, 15.4% hemicellulose, 7.1% lignin, 3.4% pectin and 3.9% fat and waxes. Based on this composition, okra has high biomethane potential.

Biochemical methane potential (BMP), according to Raposo et al.[27] and Jingura and Kamusoko[28], is a simple but reliable procedure for determining maximum methane volume produced per gram of the substrate's volatile solid and indicates rate and extent of conversion of biodegradable organics to methane in an anaerobic digestion set-up. There are both experimental and theoretical BMP methods. Although the BMP of okra has not been studied, other vegetables and food wastes have been studied using Buswell's and modified Dulong's equations[29,30] with the elemental (carbon, hydrogen, nitrogen, sulphur and oxygen) compositions of substrates[22,30,31].

Kinetic modelling is an accepted method[32] to show the specific parameters of system performance. Experimental data are used in kinetic studies and results from these studies are often applied under the same conditions to estimate operational efficiencies of scaled-up reactors. Various kinetic model types, particularly first-order kinetic models, have been successfully used to simulate anaerobic digestion processes. Akin to the phase of bacterial growth, the rate of biomethane production showed a rising limb and a decreasing limb, which were indicated by exponential and linear equations.[19,33] In the past, numerous researchers have predicted biomethane production potential using modified Gompertz, logistic and first-order kinetic models[2,10,11,34,35], as well as sigmoidal models and other statistical models[19,33,36].

The variation in the characteristics of okra waste from place to place, based on agronomical differences and storage conditions before digestion, necessitates the evaluation of its kinetic properties. Fitting kinetic functions to the cumulative methane production curves obtained from the BMP process enables information on anaerobic process performance to be gathered. This information includes: whether the maximum methane yield (Bo) was attained, the maximum rate of methane production (R_{max}), the degradation rate constant (K) and the lag phase (λ) duration.[33] The accuracy of biogas yield prediction in the model is dependent on the substrate that is used as the feedstock.

This study was motivated by the huge amount of okra waste and its perceived high biomethane potential. We assessed the biodegradability and degradation kinetics of okra waste using both Buswell's and Dulong's theoretical BMP equations, and investigated the elemental composition of the substrate to the BMP assay and used the measured BMP data in five identified growth functions (modified Gompertz, Stannard, transference, logistic and first-order models). We also determined the suitability of these models for anaerobic digestion of okra waste.

Materials and method

Substrate and inoculum characterisation

Pods (fruits) of okra (*A. esculentus*) waste were collected from Organic Farm in Centurion (Gauteng Province, South Africa) and mechanically pretreated. Inoculum from an active digester at the University of Johannesburg was degassed and acclimatised at 37 °C before use.[31] The total solids (TS), volatile solids (VS), ash content and moisture content were measured using the standard gravimetric method (Method 1684 of the US EPA for Total, Fixed and Volatile Solids in Water, Solids, and Biosolids). The carbon (C), hydrogen (H), oxygen (O), sulfur (S) and nitrogen (N) contents were determined using a CHNS elemental analyser. Elemental composition (C, H, N, S) of the samples was determined using a LECO CHNS-932 combustion analyser (TruMac, Argon, LECO Corporation, St. Joseph, MI, USA) at 1050 °C, with sulfamethazine as a standard substrate in accordance with Raposo et al.[27]. Oxygen content was calculated by assuming C + H + O + N + ash = 99.5% (on a VS basis).[37] pH was measured using a pH meter (HI 9828 Multi-parameter, Hanna Instruments). All characterisation results are shown in Table 1.

Table 1: Proximate and ultimate analyses of samples

Properties	Inoculum	*Abelmoschus esculentus*
Initial pH	8.08±0.02	8.13±0.01
Final pH	7.68±0.01	8.15±0.02
Moisture content %	98.50±0.01	92.36±4.402
Ash content %	0.03±0.00	15.87±0.006
Total solids (TS) %	1.50±0.01	7.82±0.005
Volatile solids (VS) %	1.02±0.04	6.90±0.001
VS of TS %	68±0.01	88.36±4.402
Removed VS of TS %	89.5±0.00	76.06±0.12
Carbon% TS	NT	39.30±0.012
Hydrogen% TS	NT	5.39±0.003
Oxygen% TS	NT	35.74±0.003
Nitrogen% TS	NT	3.21±0.003
C/N ratio	NT	12.24±0.003

NT, not tested

Biomethane potential

The biomethane potential of okra waste process was performed in triplicate using the BMP assay (AMPTS II, Bioprocess Control, Sweden) with 500-mL reaction bottles at Bioprocess Laboratory, Mechanical and Industrial Engineering, University of South Africa, Florida Campus (Johannesburg, South Africa) as shown in Figure 1. Each reactor was filled to 400 mL of the total volume with the addition of 27.55 g okra based on 6.90% VS, 370.94 mL of inoculum (inoculum to substrate ratio was 2:1) and 1.51 mL of distilled water. Nitrogen gas (Afrox Gas, South Africa) was used to flush out oxygen from the reactors. The reactors were operated at mesophilic temperature (37±1 °C) for 25 days. The entire test was performed as stipulated by the AMPTS II standard operation manual. Results were retrieved from the data logging platform of the reactors and used for the calculation of daily biogas production, production rate and cumulative methane production, as shown in Table 2 and Figure 2.

Figure 1: Biomethane potential assay with the data acquisition system.

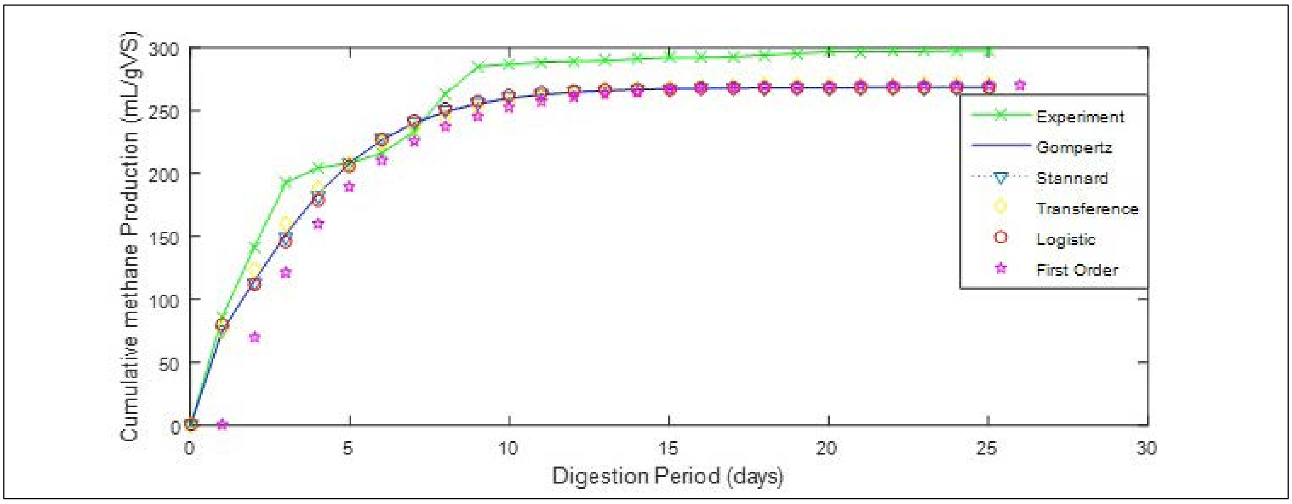

Figure 2: Cumulative biogas production based on experimental and kinetic modelling results.

Table 2: Summary of key energy production parameters

Parameter	Result
BMP (mL/gVS)	270.98
TBMP (mLCH_4/gVS)	444.48
$TBMP_{E*}$ (mLCH_4/gVS)	342.06
E* (MJ/kg) on %TS	14.63
E* (MJ/kgVS) on 88.36 %VS	12.93
BD (%)	60.97
BD_{E*} (%)	79.22
Substrate formula	$C_{14.3}H_{23.4}O_{9.8}N_1$

BMP, biomethane potential; TBMP, theoretical biomethane potential; E, energy; BD, biodegradability

Theoretical biomethane potential and biodegradability

Methane production potential and biodegradability of okra were estimated using two theoretical biomethane potential (TBMP) approaches – Buswell and modified Dulong formulae – based on okra's elemental composition.[29,30] The energy value of feedstock E* (okra) and its theoretical biomethane potential ($TBMP_{E*}$) were estimated using the modified Dulong equation. Boyles (modified Buswell) equation was used to determine the TBMP[38] and biodegradability was calculated as shown in Equations 1 to 5. TBMP was predicated based on the following assumptions[3]: ideal microbial condition and total substrate digestion; complete mixing and constant temperature; substrate composition limited to only C, H, O, N, S and output in the form of CH_4, CO_2, NH_3.

$$E* = 337(C) + 1419\ \left(H - \tfrac{1}{8}O\right) + 93(S) + 23.26(N)$$

Equation 1

$$TBMP_{E*} = \frac{E*\ (\text{base on }\%VS)}{37.78}$$

Equation 2

$$C_nH_aO_bN_c + \left(n - \tfrac{a}{4} - \tfrac{b}{2} - \tfrac{3c}{4}\right)H_2O \rightarrow \left(\tfrac{n}{2} + \tfrac{a}{8} - \tfrac{b}{4} - \tfrac{3c}{8}\right)CH_4 + \left(\tfrac{n}{2} - \tfrac{a}{8} + \tfrac{b}{4} + \tfrac{3c}{8}\right)CO_2 + cNH_3$$

Equation 3

$$TBMP = \frac{22400\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{ac}{8}\right)}{12n + a + 16b + 14c}$$

Equation 4

$$BD_{CH4} = \frac{BMP}{TBMP} \times 100$$

Equation 5

where E* is the energy value of the substrate (MJ/Kg); methane energy content = 37.78 MJ/m^3 at STP; CHONS = carbon, hydrogen, oxygen, nitrogen, sulfur (% TS); TBMP is the theoretical biomethane potential at STP ; and BD_{CH4} is the anaerobic biodegradability (%).

Kinetic models for biogas production

Non-linear regression analysis was performed using the curve-fitting tool in Matlab R2015b to evaluate the growth functions (modified Gompertz, Stannard, transference, logistic and first-order kinetic models) shown in Equations 6 to 10. The average measured cumulative methane production was used to evaluate the models; the model parameters and the goodness of fit are shown in Table 2 and Figure 2.

Modified Gompertz
$$B = B_0 Exp\left\{- Exp\left[\frac{R_{max} \cdot e}{B_o}(\lambda - t)\right] + 1\right\}$$
Equation 8

Stannard
$$B = B_0\left\{1 + Exp\left[-\frac{(1+kt)}{p}\right]\right\}^{-p}$$
Equation 9

Transference
$$B = B_0\left\{1 - Exp\left[\frac{R_{max}}{B_o}(t - \lambda)\right]\right\}$$
Equation 10

Logistic
$$B = \frac{B_o}{\left\{1 + Exp\left[\frac{4R\ max}{B_o}(t - \lambda) + 2\right]\right\}}$$
Equation 11

First-order
$$B = B_0(1 - Exp(-kt))$$
Equation 12

where B is cumulative specific methane production (mL/gVS); B_o is maximum specific methane production potential (mL/gVS); R_{max} is the maximum specific methane production rate (mL/gVS- d); e is Exp(1)=2.718282; λ is the lag phase in days; k is the methane production rate constant (day^{-1}); t is digestion time (days); and p is slope of growth.

The kinetics of biogas production were evaluated using the five growth functions to determine the following parameters: B_o, B_p, k, λ, p, R^2, Adjusted R^2, R_{max} and root mean square error (RMSE). The entire experiment was performed in triplicate and the average of the three values was used. Minitab 15 was used for all statistical analyses and all inferences are at a 95% confidence.

Results and discussion

The ultimate and proximate properties of okra waste are shown in Table 1. A mass of 27.55 g was determined based on 7.8157 %TS and 6.8945 %VS. Although a high substrate VS/TS ratio of 88.36% was recorded, 76.06% of the substrate was removed during the anaerobic digestion process. This finding is in agreement with Li et al.[31] who reported a high VS/TS to be desirable for biogas yield. The waste showed a C/N ratio of 12.24, which was outside the ideal range of 15–30, thus necessitating co-digestion or nutrient enrichment.[31]

The experimental BMP assay gave a digestion period of 25 days, as shown in Figure 2. Okra waste resulted in a methane yield of 270.98 mL/gVS, which concurs with other reports of low yields from lignocellulosic vegetable wastes.[22,31] Theoretical biomethane potential (TBMP) and

Table 3: Kinetic parameters of average cumulative methane production curves

Parameter	Modified Gompertz	Stannard	Transference	Logistic	First-order
Measured biogas yield, $B_{(t)}$ (mL/gVS)	270.98	270.98	270.98	270.98	270.98
Predicted biogas yield, $B_{(P)}$ (mL/gVS)	268.38	267.99	270.89	267.50	270.15
Difference between $B_{(t)}$ and $B_{(p)}$ (%)	0.95	1.1	0.03	1.28	0.31
B_o (mL/gVS)	268.4	268.0	271.1	267.5	270.3
R_{max} (mL/gVS)	39.93	–	77.2	34.38	–
Lag phase, λ (days)	0.872	–	0.143	1.24	–
Degradation rate, K (per day)	–	1.449	–	–	0.2994
P	–	3.269	–	–	–
R^2	0.963	0.957	0.983	0.946	0.982
Adjusted R^2	0.96	0.953	0.982	0.941	0.981
RMSE	13.67	14.7	9.209	16.54	9.378

biodegradability (BD_{CH4}) calculated using Equations 1 to 5 using elemental and energy content of the substrate are shown in Table 2. TBMP based on elemental composition (444.48 mL/gVS) was higher than that obtained based on energy content (342.06 mL/gVS). BD_{CH4} based on elemental composition (60.97%) was lower than that based on energy content (79.22%). Raposo et al.[27] reported that BD_{CH4}<70% is considered an outlier or invalid. In view of this finding, TBMP based on energy content better satisfied the criterion. The low BD_{CH4} seen in elemental TBMP is consistent with the biodegradability of lignocellulosic vegetables.[22,31]

The measured and predicted methane production results, as well as the determined parameters, are shown in Figure 2 and Table 2. The cumulative measured biogas was 270.8 mL/gVS; the models predicted cumulative biogas to be 267.38, 267.99, 270.89, 267.50 and 270.15 mL/gVS, respectively, for modified Gompertz, Stannard, transference, logistic and first-order models. These values are consistent with the assertion of Raposo et al.[27], who recommended that the difference between B_o and B_t should not be more than 10%, above which this kinetic model is deemed invalid for predicting anaerobic digestion processes.

The lag phase (λ) of the growth functions, which is the time required for bacteria to adapt and start biogas production, is given in Table 3 and Figure 2. The values are 0.872, 0.143 and 1.24 for modified Gompertz, transference and logistic models, respectively. The low λ values found in this study are in line with the report of Talha et al.[39], who stated that lower lag phase is dependent on the activeness of the adapted inoculum and biodegradability of the organic part of the okra waste.

Most lignocellulosic substrates have cellulose as their main polymer component (about 68% in the case of okra). The hydrolysis rate of cellulose is normally the rate-limiting step, and the biomethane production rate is denoted by *k*.[40,41] The *k*-value of substrates can be determined via product formation (biomethane production or VFAs) and substrate depletion (VS, COD or DOC) methods.[42] In this study, biomethane production (the product formed) was used to compute the *k*-values of both Stannard and first-order models of 1.449/day and 0.2994/day, respectively. The *k*-values obtained were both high and positive, which, according to Dudek et al.[41], could be because of the higher bioavailability of cellulose, which results in a faster rate of biogas production.[34,35] This observation is in agreement with that of Veeken and Hamelers[43], namely that biomethane production represents the hydrolysis rate of bioavailable substrate which decreases with decreasing VS and can be best described with first-order kinetics.

Transference and first-order models best predicted okra waste digestion, with a prediction difference of 0.03% and 0.31%, respectively. This finding is consistent with the report of Kafle and Chen[36], who showed that the first-order kinetic model was found to be the best model for predicting BMP. Li et al.[19] reported that the transference model performed better than the modified Gompertz model. The statistical indicators of model fitness (as shown in Table 3), ranged from 0.946 to 0.983, 0.941 to 0.982 and 9.209 to 16.54 for R^2, Adj. R^2 and RMSE, respectively. In line with the report of Budiyono and Sumardiono[35], an RMSE value of <10 shows good model prediction. Based on this criterion, only transference and first-order kinetic models were within the accepted limit.

Conclusions

Experimental biomethane potential, biodegradability and degradation kinetics of okra waste were evaluated in this study using five growth functions. It was also shown that both energy content and elemental composition evaluation methods could be reasonably used to calculate TBMP and BD_{CH4} of okra waste. The goodness of fit, good predicted methane yield and lowest percentage prediction difference as observed showed that both transference and first-order models performed better than the other models evaluated. The positive kinetic constant and lower lag phase confirmed the high rate of degradation. Based on the goodness of fit, the logistic model performed the worst. Based on the substantial cumulative BMP yield of this novel substrate, further studies aimed at improving its biodegradability will be desirable.

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Authors' contributions

S.N.U. was responsible for conceptualisation; methodology; data collection; data analysis; validation; initial draft. E.C.C. was responsible for project leadership and management; writing revisions and student supervision.

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