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Assessment of models for anaerobic biodegradation of a model bioplastic: Poly(hydroxybutyrate-co-hydroxyvalerate)

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A B S T R A C T

Kinetic models of anaerobic digestion (AD) are widely applied to soluble and particulate substrates, but have not been systematically evaluated for bioplastics. Here, five models are evaluated to determine their suitability for modeling of anaerobic biodegradation of the bioplastic poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV): (1) first-order kinetics with and without a lag phase, (2) two-step first-order, (3) Monod (4) Contois, and (5) Gompertz. Three models that couple biomass growth with substrate hydrolysis (Monod, Contois, and Gompertz) gave the best overall fits for the data ($R^2 > 0.98$), with reasonable estimates of ultimate CH_4 production. The particle size limits of these models were then evaluated. Below a particle size of 0.8 mm, rates of hydrolysis and acetogenesis exceeded rates of methanogenesis with accumulation of intermediates leading to a temporary inhibition of CH_4 production. Based on model fit and simplicity, the Gompertz model is recommended for applications in which particle size is greater than 0.8 mm.

1. Introduction

Biodegradable plastics offer a more environmentally sustainable alternative to traditional plastics for a variety of applications, but management of these materials at end-of-life is critical for sustainability. Uncontrolled degradation of organic materials in anaerobic environments leads to release of methane (CH_4) into the atmosphere; by contrast, controlled anaerobic biodegradation via anaerobic digestion (AD) can enable efficient recovery of CH_4 for use as a fuel or feedstock. AD has been widely studied for a variety of solid organic wastes (Ge et al., 2016; Vavilin et al., 1996, 2008; Xu et al., 2015), but emerging bioplastics, such as polylactic acid and poly(hydroxyalkanoates) (PHAs), have received little attention. This work addresses this deficiency by evaluating kinetic

models previously used for modeling of solid substrate degradation to determine their applicability for modeling of the anaerobic biodegradation of a model bioplastic: Poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV), a commercially available PHA with mechanical properties similar to those of polypropylene (Miller et al., 2015; Tsui et al., 2013).

2. Kinetic models of solid substrate degradation

Several steps must be considered when modeling the biodegradation of solid substrates, such as bioplastics, including: (1) initial colonization of a surface and biofilm formation, (2) diffusion of enzymes to the surface, (3) hydrolysis, (4) diffusion and degradation of hydrolysis products, (5) conversion of hydrolysis products into intermediates, and (6) conversion of intermediates to CH_4 by methanogenic biomass. To develop practical models with a small number of fitting parameters, a rate-limiting step is typically

Table 1
Expressions used to model bioplastic degradation.

Model	Description	
Monod	Parameters [†]	μ_m : maximum specific growth rate [T^{-1}] K_S : Monod constant [$M_S L^{-3}$] X : biomass concentration [$M_X L^{-3}$] Y : biomass yield [$M_X M_S^{-1}$]
	Rate Limiting Step	Acetoclastic methanogenesis
	Lag Time	Yes
	Assumptions [‡]	Rapid hydrolysis, rate determined by microbial growth controlling methanogenesis (Vavilin et al., 2008)
First-Order	Parameters [†]	k : maximum hydrolysis rate constant [T^{-1}]
	Rate Limiting Step	Substrate hydrolysis
	Lag Time	No
	Assumptions [‡]	Rate and substrate concentration are proportional, rate limited by surface availability of the substrate (Vavilin et al., 2008)
First-Order With Lag	Parameters [†]	k : maximum hydrolysis rate constant [T^{-1}] λ : Initial lag time to substrate hydrolysis [T]
	Rate Limiting Step	Substrate hydrolysis
	Lag Time	Yes
	Assumptions [‡]	After initial lag, reaction is first-order
Two-Step With Lag	Parameters [†]	k_H : first-order kinetics constant of substrate degradation into VFAs [T^{-1}] (first step) k_{VFA} : first-order kinetics constant of VFA degradation into CH_4 [T^{-1}] (second step)
	Rate Limiting Step	Two potentially rate determining steps: hydrolysis to VFAs and conversion of VFAs to CH_4
	Lag Time	Yes
	Assumptions [‡]	Two first order reactions describing hydrolysis/acetogenesis and acetoclastic methanogenesis (Brulé et al., 2014)
Contois	Parameters [†]	$\rho_m H$: maximum hydrolysis rate [$M_S M_X^{-1} T^{-1}$] K_C : Contois constant [$M_S M_X^{-1}$] X_H : hydrolytic (acidogenic) biomass concentration [$M_X L^{-3}$] Y_H : hydrolytic biomass yield [$M_X M_S^{-1}$]
	Rate Limiting Step	Substrate hydrolysis coupled to growth of hydrolytic biomass
	Lag Time	Yes
	Assumptions [‡]	A single parameter can represent saturation of both substrate and biomass (Vavilin et al., 2008)
Gompertz	Parameters [†]	R : maximum rate of CH_4 production [$L_{CH_4}^3 M_S^{-1} T^{-1}$] λ : lag time to CH_4 production [T]
	Rate Limiting Step	Microbial growth rate (not explicitly limited by substrate hydrolysis)
	Lag Time	Yes
	Assumptions [‡]	Not constrained by availability of substrate, direct relationship between substrate utilization, methanogenic biomass growth, and CH_4 production (Zwietering et al., 1990; Lay et al., 1998)

[†] All models include the parameter P_m , the maximum conversion of substrate into CH_4 [$L_{CH_4}^3 M_S^{-1}$].

[‡] All models neglect biomass decay and this work assumes that the initial CH_4 volume, $P_0 = 0$.

assumed. Table 1 summarizes the key models developed to date, fitting parameters, and key assumptions, including rate-limiting steps (Brulé et al., 2014; Lay et al., 1998; Vavilin et al., 2008; Zwietering et al., 1990). Of particular interest are the Contois model and the Gompertz model. The Contois model couples hydrolysis to growth of the hydrolytic biomass with a single fitting parameter (Vavilin et al., 2008). A recent theoretical derivation indicates that this model can account for colonization, diffusion, and uptake of hydrolysis products (Wang and Li, 2014). The Gompertz model accounts for different stages in the conversion of substrate to CH_4 and incorporates a lag time parameter, a maximum rate in the linear region of the CH_4 generation curve, and the ultimate capacity of CH_4 production (Lay et al., 1998; Zwietering et al., 1990).

An important consideration for all of the models in Table 1 is particle size, the key determinant of specific surface area (SSA). SSA can be estimated from particle size, then substituted for the available substrate, S , in the Monod and Contois equations of Table 1 (Kong et al., 2003). The maximum rate of hydrolysis, ρ_{mH} , then becomes dependent upon SSA.

Understanding how particle size, and therefore surface area, influences the rate of anaerobic degradation is also important for biomaterials management decisions, such as the desirability of mechanical pretreatment to reduce particle size. Smaller particle size can increase hydrolysis rates and accelerate AD (Kong et al.,

2003; Vavilin et al., 2008; Yeh et al., 2010), but the rate of hydrolysis must balance the rate of uptake of volatile fatty acids (VFAs). If the rate of hydrolysis exceeds the rate of uptake of VFAs, VFAs accumulate and methanogenesis can be inhibited, and the models of Table 1 would no longer apply (Vavilin et al., 2004). Smaller particles with high surface area and high rates of hydrolysis could lead to this condition.

The following sections describe anaerobic PHBV degradation experiments and model fits for CH_4 production using the kinetic expressions of Table 1. The aim of this work is to identify a model that provides insights into the mechanisms of PHBV biodegradation and can be incorporated into design models that simulate and predict PHBV degradation in AD applications.

3. Materials and methods

3.1. Materials

3.1.1. PHBV and positive control

PHBV and Positive Control: The bioplastic substrate used in these experiments was PHBV, a biodegradable aliphatic polyester with the chemical formula: $[COCH_2CH(CH_3)O]_m[COCH_2CH(C_2H_5)O]_n$. Fine PHBV powder (ENMAT Y1000) and coarse PHBV pellets (ENMAT Y1000P) were obtained from TianAn Biologic Materials

Co., People's Republic of China. Injection molded PHBV samples made from the PHBV pellets were fabricated per the process of Srubar et al. (Srubar et al., 2012) and were rectangular prisms, 31.25 mm × 6.2 mm × 2.1 mm in dimension. In order to investigate the effects of different particle sizes, PHBV pellets were ground in a standard coffee grinder and sieved to obtain 3 lots of ground pellets in particle sizes shown in Fig. 1, Panel (a). Un-ground PHBV pellets and PHBV powder were used to provide higher and lower particle sizes to the ground samples. The measured density, ρ , of bulk PHBV was 1.25 g/cm³.

Avicel® PH105 microcrystalline cellulose was used as a positive control (ASTM, 2002). The average particle size was 20 μ m, with particle diameters ranging from 5–30 μ m and a length-to-width aspect ratio of 2–3 (Katdare and Chaubal, 2006).

3.1.2. Particle characterization

In order to assess the particle surfaces and diameter of the different samples, the ground PHBV pellets and powder were imaged using optical microscopy with a Nikon SMZ800 Optical Microscope. Images were calibrated with a micrometric graticule (Silicone Test Specimen, EMS, Catalog # 79502) with 500 μ m coarse spacing and fine spacing of 10 μ m.

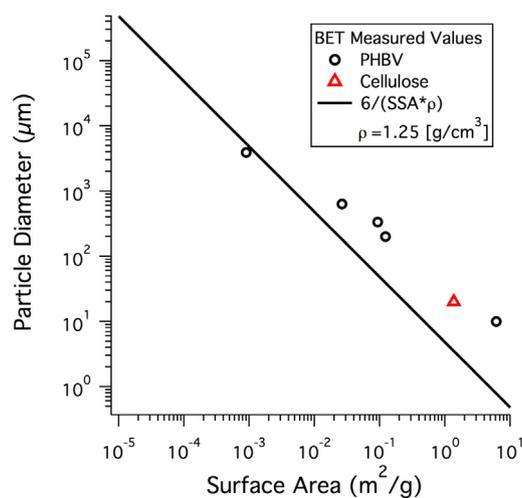
The Brunauer, Emmett and Teller (BET) method was used to determine the SSA of the different particle size fractions using a

Micromeritics ASAP 2020 Surface Area and Porosity Analyzer and nitrogen (N₂) adsorption. Approximately 2 g of powdered sample was used for the analysis. Prior to measuring the SSA, samples were degassed at 110 °C for 12 h, weighed, and then loaded into the BET for analysis. The analysis conditions were for the full isotherm for BET and BJH porosity.

For samples too large for BET analysis, sample dimensions were measured with Mitutoyo Model CD-6°C Calipers for larger sample dimensions (greater than 25 mm) and with a Mitutoyo Model 293–344 Micrometer for smaller sample dimensions. Three locations along a sample plane were selected for each dimension and averaged to calculate sample surface area and volume.

Fig. 1 shows the surface area of the different particle size fractions (Panel a) and the BET surface area and calculated diameter (Panel b). The solid line in Fig. 1a shows the relationship that would be expected for spherical, non-porous particles of a known density ($\rho = 1.25$ g/cm³). The measured SSA leads to a smaller particle size than would be expected.

The PHBV particles are not spherical and have additional surface roughness, therefore this deviation from theory can be expected. BET is a surface area measurement inferred from N₂ adsorption on the particle surfaces, and is expected to overestimate the surface area (SA) available for enzymatic binding. Despite this limitation, the BET method provides a quantifiable assessment



(a)

PHBV particle size and BET surface area and particle diameter

Sample	Sieve No. [Mesh]	Particle Size [μ m]	BET SSA [m ² /g]	BET Diameter [μ m]
PHBV:				
Injection Molded	NA	6,584 [†] , *	NA	NA
Pellets	NA	3900 [†]	0.0009	5333
Ground 1	-20 + 40	420-840	0.0264	182
Ground 2	-40 + 60	250-420	0.093	52
Ground 3	-60 + 100	150-250	1.222	39
Powder	NA	10 [‡]	6.1445	0.8
Cellulose	NA	20 [‡]	1.3651	2.9

[†] Measured via caliper.

[‡] Reported.

* Calculated from rectangular prism, 31.25 mm × 6.2 mm × 2.1 mm.

(b)

Fig. 1. Particle characterization for PHBV with different particle sizes: (a) Particle diameter versus surface area as measured by BET or caliper for the PHBV size fractions and the cellulose control. The solid line indicates the surface area-diameter relationship expected for non-porous spherical particles. (b) Table of particle size fractions and BET surface area and particle diameter. Particle size is listed from coarsest (pellets) to finest (powder).

of the SSA of each size fraction that can be used to assess how SA impacts CH₄ production rates and lag times.

3.2. Experimental setup

Methane production was continuously measured using the AMPTS II from Bioprocess Control. Experiments to assess models for anaerobic biodegradation were performed using injection molded bioplastic samples and were tested in a separate experimental group from the samples used to evaluate the dependence of CH₄ production on particle size. For each experimental group, negative controls (inoculated media) were run in triplicate. Microcrystalline cellulose (Avicel[®] PH105) was used as a positive control. The positive control was judged “fully biodegraded” when the CH₄ produced was equal to or greater than 80% of the theoretical CH₄ production potential (ThCH₄) (Mezzanotte et al., 2005). Stoichiometry was used to calculate the theoretical CH₄ production for a known mass of PHBV (Buswell and Mueller, 1952; Ryan, 2016). Briefly, the chemical oxygen demand (COD) of PHBV and the cellulose control were calculated as 1.69 g COD/g for PHBV and 1.19 g COD/g for cellulose. COD was then used to calculate the theoretical methane production from PHBV, 672 mL/gPHBV, which was compared to the measured CH₄ production and used to calculate the extent of biodegradation of the PHBV over time. To ensure equal COD loading between the reactors: (1) for model evaluation PHBV was added to each test reactor at 0.5 g, and microcrystalline cellulose (positive control) was added at 0.68 g; (2) for evaluation of surface area effects, 1.17 g of the different PHBV size fractions was added to each test sample reactor, and 1.7 g of microcrystalline cellulose to each positive control reactor. These amounts give an inoculum to substrate ratio of (1) 1.1 for model evaluation and (2) 0.5 for particle size evaluation; both values are within a desirable range for optimal CH₄ production (Boulanger et al., 2012; Liu et al., 2009). Duplicates were analyzed of each size fraction and the positive control (cellulose).

Each sample was placed in 500-mL media bottles, which were filled with 500 mL of inoculated anaerobic media, retaining a 100 mL gas headspace. The bottles were stoppered and the headspace was sparged with 70:30 N₂/CO₂ before and during filling to maintain anaerobic conditions in the sample container. The inoculated anaerobic media was continuously stirred during filling with a magnetic stirrer and gas agitation from being sparged with N₂/CO₂. Reactors were filled sequentially; media was pumped continuously with a bellows metering pump (GRI 14251-003). Once filled, the bottles were connected with viton tubing to the Automated Methane Test Setup (AMPTS) gas measuring setup. Bioreactors were maintained at 37 °C by keeping them submerged in a water bath.

Gas sampling “T’s” inline after the reactor headspace and before the carbon dioxide (CO₂) traps were used to sample the gas composition during degradation using gas chromatography. Gas sampling “T’s” were obtained through BioProcess Control via SeaHold LLC. These glass sampling ports were fit with 13 mm butyl rubber/Teflon stoppers (Kimble Chase, Part No. 73811T-13), sealed with 13 mm tear-off aluminum crimp seals. CO₂ traps were added inline and filled with 3 M sodium hydroxide with a thymolphthalein pH indicator. CH₄ was measured volumetrically in calibrated reservoirs in increments of approximately 10 mL. Data were calibrated, normalized, and corrected for negative control gas volume using Matlab. Reactors were enabled for overhead mixing with a stir speed of 50 rpm and 4 h mixing cycles: 30 min of mixing followed by 330 min of quiescence. These parameters were selected based on prior work indicating that aggressive mixing may inhibit microbial adhesion on the bioplastic substrates (Gartiser et al., 1998; Morse, 2010).

The gas compositions of the reactor headspaces were measured at 3 and 7 days, then every week until the 4th week, at which time CH₄ production had slowed. The final reading was obtained at 6 weeks, after the CH₄ production had plateaued. The reactor effluent, 2 mL, was collected twice during the period of active degradation (while mixing, at 1.5 and 3 weeks) as well as at the beginning and end of each experiment and measured for pH. Mineral media (2 mL) was added to each reactor after sampling to prevent errors in gas production measurement due to liquid volume.

After CH₄ production from the samples used for model evaluation had plateaued, new PHBV samples and positive controls were added to their respective bioreactors to evaluate any changes that might occur in the biodegradation of the bioplastic due to microbial adaptation to the substrate. All other experimental conditions were kept the same. The Gompertz model was used to compare the impact of substrate acclimation to as-collected inoculum.

3.3. Anaerobic media and inoculum

The anaerobic media used for these experiments was based primarily on standard solutions as described in ASTM D5210-92 (ASTM, 2007; Owen et al., 1979), with modifications to concentrations based on other studies (Kenealy and Zeikus, 1981; Shelton and Tiedje, 1984; Wu et al., 1991). The following 4 concentrated stock solutions were used: (S1) resazurin, 0.5 g/L; (S2) KH₂PO₄, 69 g/L, K₂HPO₄, 88 g/L, NH₄Cl, 100 g/L; (S3) MgCl₂·6H₂O, 60 g/L, CaCl₂·2H₂O, 45 g/L, FeCl₂, 12 g/L, MnCl₂·4H₂O, 400 mg/L, CoCl₂·6H₂O, 400 mg/L, NiCl₂·6H₂O, 50 mg/L, CuCl₂, 50 mg/L, ZnSO₄·7H₂O, 105 mg/L, H₃BO₃, 50 mg/L, Na₂MoO₄·2H₂O, 50 mg/L, Na₂SeO₃, 10 mg/L; and (S4) Na₂S·9H₂O 50 g/L. These 4 stock solutions were used in the same proportions as in ASTM D5210 to make the media, following the preparation instructions included in the standard. Bicarbonate (NaHCO₃) was added to give a final concentration of 50 mM in the media. The anaerobic media was continuously stirred and sparged with N₂/CO₂ 70:30 (Praxair certified standard, NI CD30C-K) during the addition of the inoculum to maintain anaerobic conditions. Anaerobic digester sludge was added as an inoculum to the anaerobic mineral media as 10 vol.% of the total volume.

Anaerobic sludge from digesters treating municipal waste water was selected as the inoculum for these experiments based on existing standards and prior results indicating that it would provide a reliable anaerobic environment to evaluate PHBV degradation (ASTM, 2007; Morse, 2010; Ryan, 2016). Anaerobic inocula were collected from the anaerobic digesters at the San Jose Waste Water Treatment Plant in 4 L sample containers. These samples were stored in the dark at 37 °C for two weeks prior to initiating experiments in order to reduce background activity from the sludge. Prior to testing, the sludge was screened through a 1 mm mesh sieve followed by a single layer of cheese cloth in order to reduce grit and large solids that may have interfered with the experiment. This step was performed immediately before adding the inoculum to the anaerobic media. Anaerobic digester sludge and inoculated media were characterized for COD, pH, suspended solids (SS), volatile solids (VS), and alkalinity in accordance with standard methods (American Public Health Association et al., 1998). The values for both sets of experiments are given in Table 2.

3.4. Biogas composition

Samples analyzed for biogas composition were collected using a 1 mL gas tight syringe (VICI, Pressure Lok[®] Series A-2 syringe, Product No. 050033) with a sideport gas sampling needle (VICI, Product No. 943052). CH₄, CO₂ and N₂ were measured using gas chromatography on a Gow-Mac 580 Series gas chromatograph equipped with a thermal conductivity detector (TCD). The gasses

Table 2

Characteristics of anaerobic digester sludge inoculum and inoculated media for kinetic evaluation of models and surface area experiments.

Property	Kinetic models		Surface area		
	Sludge	Media [†]	Sludge	Media [‡]	
pH	7.56	7.51	7.68	7.52	
Alkalinity [‡]	4,100 ± 110	–	4,400 ± 110	–	mg CaCO ₃ /L
COD (Total)	13,470 ± 265	3,800 ± 190	17,000 ± 250	1,800 ± 100	mg COD/L
COD (Solids)	1.4 ± 0.1	–	–	–	g COD/g VSS
SS	16.8 ± 0.2	2.0 ± 0.3	5.6 ± 0.2	1.8 ± 0.2	g SS/L
VS	11.4 ± 0.1	1.1 ± 0.2	3.8 ± 0.1	1.1 ± 0.2	g VS/L

[†] Media is inoculated with 10 vol% anaerobic digester sludge.

[‡] Alkalinity samples were titrated to pH 4.5 (American Public Health Association et al., 1998) and measured without biosolid removal.

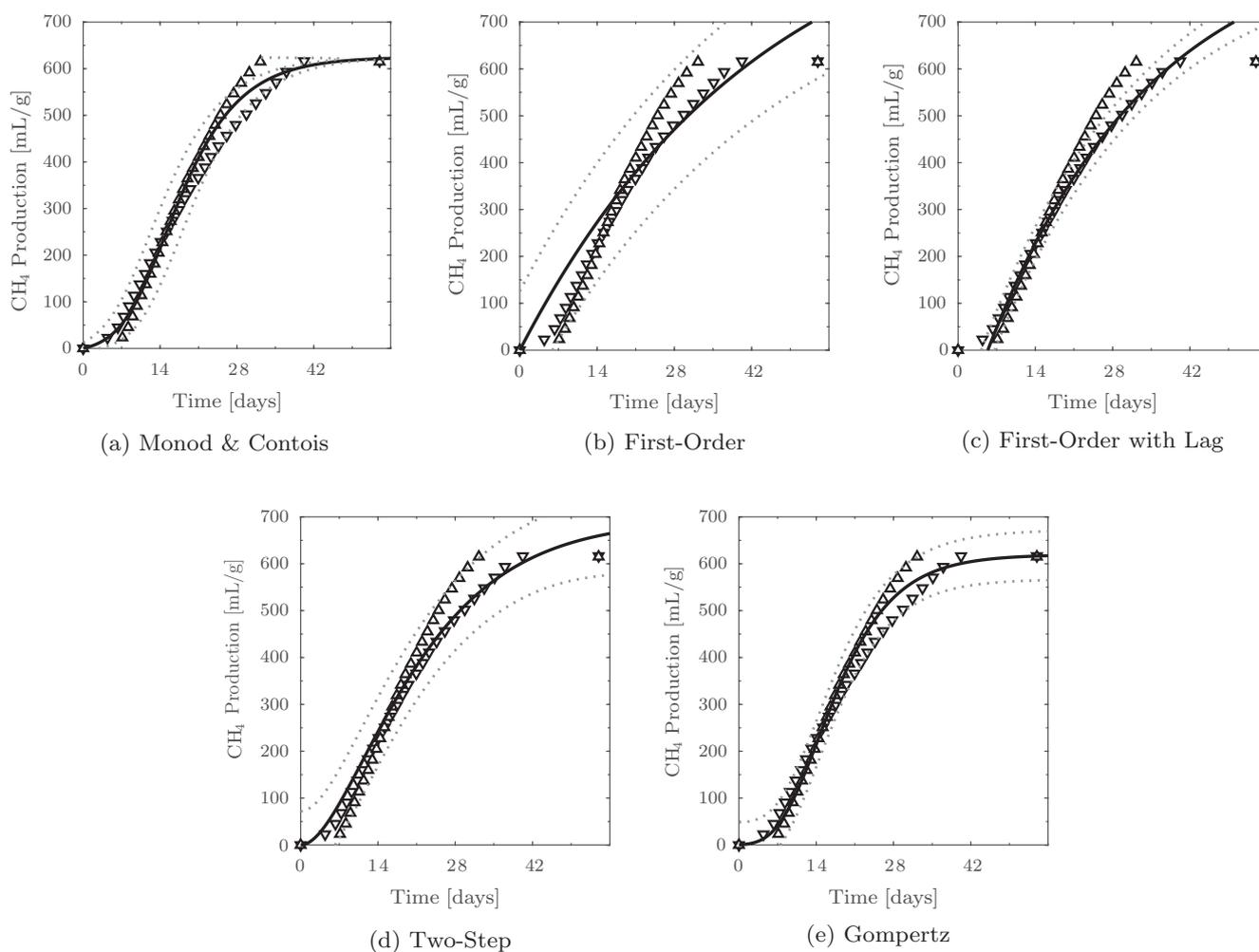


Fig. 2. Kinetic models applied to CH₄ production from PHBV: data markers (Δ , ∇) show the measured data from two injection molded PHBV samples and the lines show the model fit (–) and 95% prediction bands (···) for each data set.

were separated at 45 °C on a 8' × 1/8" HayeSep Q, 80/100 mesh micropacked column. (He) was used as a carrier gas at a flow rate of 30 mL/min. The temperature of the injector and detector was kept at 120 °C. The volume collected of all samples and standards was 0.6 mL. Prior to injection, the gas in the syringe was compressed to the 0.5 mL injection volume, rapidly equilibrated with atmospheric pressure by toggling the valve on the syringe. The sample was then immediately injected into the gas chromatography (GC) sampling port. The standard run time was 4 min. Details on calibration and method development are as described by Ryan (2016).

3.5. Evaluation of kinetic models

Matlab 2015b was first used to fit CH₄ production from negative control samples. This fit was used to generate a lookup table to subtract out the background CH₄ generated by the inoculum from positive control and sample data.

The kinetic models of Table 1 were then fit to CH₄ gas production data pooled from two injection molded PHBV samples using the Curve Fitting application in Matlab 2015b, with non linear least squares and the Trust-Region algorithm. Upper and lower 95% confidence and prediction intervals and goodness of fit (R^2) were cal-

culated for all fit parameters. Matlab was also used to solve the system of linear equations and extract the kinetic parameters from the fits for the Monod and Contois models. The criteria used to evaluate the models were: (1) ability to accurately predict ultimate CH₄ production (P_m), (2) overall goodness of fit (R^2), (3) the 95% confidence interval of the fit parameters, and (4) the ability to accurately describe the CH₄ production over time.

The following experimental parameters were used for model evaluation and CH₄ production calculations: (1) $S_0 = 1.58$ g COD/L for PHBV, (2) PHBV was measured to be greater than 99% volatile solids (for CH₄ production calculations), (3) post-sieved, filtered volatile solids were assumed to be biosolids such that $X_0 = 1.05$ g VS/L (Bullock et al., 1996) (used for the Contois model), and (4) the theoretical value for P_m (PHBV) at 37 °C is 672 mL CH₄/g VS.

IgorPro 6.37 was used to analyze the maximum rate and lag time in the surface area experiments. Rate and lag time were calculated from the derivative of CH₄ production with time, as the peak value of the derivative (maximum rate) and the time to initiation of CH₄ production. These values were used to model how surface area affects degradation using non-linear regression in Igor Pro. This model generated maximum CH₄ production rate, R , and lag time for CH₄ production, λ , which were then used as inputs into the Gompertz model.

4. Results and discussion

4.1. Kinetic models of PHBV biodegradation

Fig. 2 shows the different models applied to CH₄ gas production data from the two rectangular prism samples of injection-molded PHBV. Table 3 summarizes the resultant fit parameters and goodness of fit for the various models. While all fit R^2 values were greater than 0.89, not all models were able to accurately capture the features of the CH₄ production curves. In particular, the first-order overestimated the ultimate CH₄ produced, and over-predicted initial CH₄ production. Adding the lag time factor into the first-order equation significantly improved the model fit for the initial CH₄ production and rate constant, but still overestimated ultimate CH₄ yield.

The Monod, Contois, two-step first-order, and Gompertz models provided good fits for CH₄ production. The Monod, Contois, and Gompertz models provided the best fit for the data as determined by comparing R^2 , the confidence intervals of the fit parameters, and

the ability to predict ultimate CH₄ production. The convergence of both the Monod-Contois fit and the two-step fit were sensitive to initialization values. The two-step model yielded wider 95% confidence intervals and prediction bands than the Monod, Contois, and Gompertz models and slightly over predicted CH₄ production. Overall, the two-step model was less accurate in capturing the CH₄ production over time than the Monod, Contois, and Gompertz models. However, it is notable that the fit values for k_H and k_{VFA} in the two-step are very similar. If a microbial biofilm is limiting diffusion of hydrolysis products to methanogenic biomass, the hydrolysis-limiting assumption often made for particulate substrates may not be accurately addressing the complexity of this system.

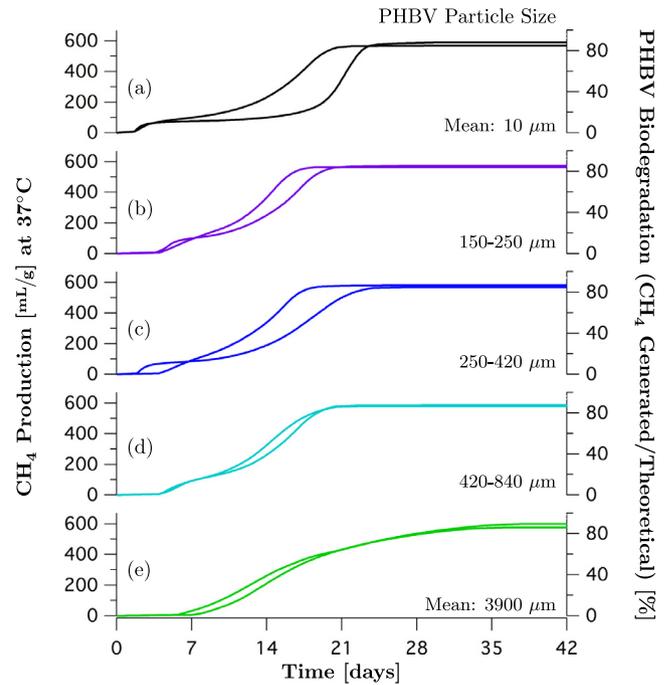


Fig. 3. CH₄ production and extent of biodegradation of PHBV with different particle sizes over time. Temporary inhibition of methanogenesis for particle sizes less than 840 μm is attributed to the accumulation of VFAs caused by the rate of hydrolysis and acetogenesis exceeding that of methanogenesis. As particle size decreases from bottom (e) PHBV pellets to top (a) PHBV powder, this effect becomes more pronounced.

Table 3
Model fit parameters for anaerobic biodegradation of PHBV.

Model	Parameter [†]	R^2 Fit
Monod	P_m $\left[\frac{\text{mLCH}_4}{\text{gPHBV}}\right]$ 625 ± 10	K_s $\left[\frac{\text{gCOD}}{\text{L}}\right]$ 2.9 ± 0.1
First-Order	1000 ± 400	X_0/Y $\left[\frac{\text{gCOD}}{\text{L}}\right]$ 0.02 ± 0.03
First-Order with lag	897 ± 60	λ [days] 5.4 ± 0.4
Two-Step	695 ± 110	k_{VFA} $\left[\frac{1}{\text{day}}\right]$ 0.087 ± 4.4
Contois	625 ± 10	K_c $\left[\frac{\text{gCOD}}{\text{gVS}}\right]$ 0.02 ± 0.001
Gompertz	630 ± 20	X_{OH}/Y_H $\left[\frac{\text{gCOD}}{\text{L}}\right]$ 0.02 ± 0.01
		λ [days] 6.4 ± 0.5

$S_0 = 1.58$ gCOD/L, 1gPHBV ≈ 1gVS, $X_0 = 1.05$ gVS/L. At 37 °C if 100% of the PHBV is converted to CH₄, $P_{m,100\%} = 672$ mLCH₄/gPHBV.

[†] Fit from two pooled data sets of CH₄ production from injection molded PHBV, ±95% confidence interval (from model fit and measurement error).

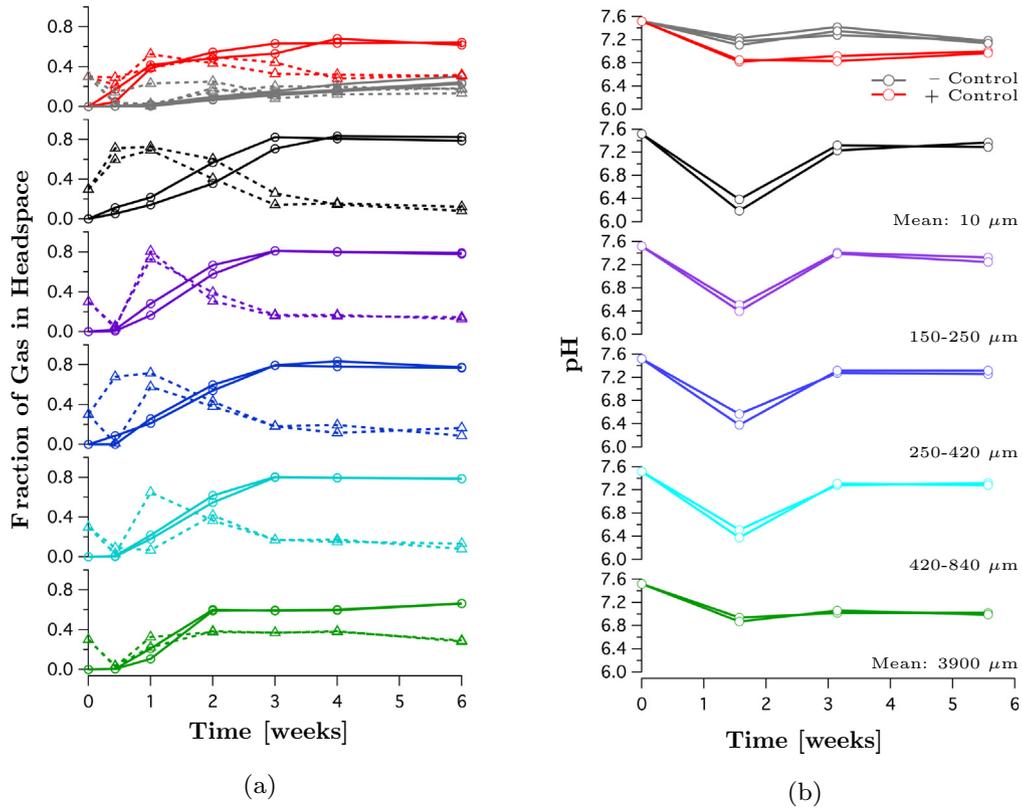


Fig. 4. Biodegradation patterns for differing size particles of PHBV (a) headspace gas composition (b) pH.

Even though the fit form for the Monod and Contois is the same, the additional dependence of the Contois equation on biomass concentration leads to a slightly different interpretation of the model parameters. The derivation of Wang and Li (Wang and Li, 2014) indicates that the Contois equation reduces to the Monod equation for those cases in which suspended cells have access to the solid substrate surface. Both constants, K_S and K_C , share the physical meaning in that smaller values suggest higher affinities for attached microorganisms to the substrate surface. Thus, the low value obtained for K_C (Table 3) indicates a high affinity for the bioplastic surface. This hypothesis is supported by scanning electron microscope (SEM) images that showed microbes attached to the bioplastic surface after 10 days of anaerobic degradation and a gentle DI water rinse.

Because both the Monod and Contois models are explicitly dependent on the availability of substrate, rate constants can be defined by the surface area to volume ratios of the initial substrate, and by changes in this ratio over time (Vavilin et al., 2008). This surface sensitivity could be extended to bioplastic and biocomposites of different sizes and dimensions, similar to investigations by Gutierrez-Wing et al. for PHBV films (Gutierrez-Wing et al., 2010). The theoretical applicability of the surface derived model for the Contois equation (Wang and Li, 2014) and the goodness of fit of both the Contois and Gompertz models, makes these two

models the most suitable for predicting AD CH₄ production from PHBV.

When the Gompertz model was applied to methane production from PHBV samples in the acclimated reactors, minimal effects due to microbial adaptation to the substrate were observed. The values for the non-acclimated reactors are given in Table 3 as: $P_m = 630 \pm 20 \left[\frac{\text{mLCH}_4}{\text{gPHBV}} \right]$, $R = 28 \pm 1.5 \left[\frac{\text{mLCH}_4}{\text{gPHBV} \cdot \text{day}} \right]$, and $\lambda = 6.4 \pm 0.5 [\text{days}]$. The fit for CH₄ production from PHBV-adapted bioreactors gave values of $P_m = 626 \pm 14 \left[\frac{\text{mLCH}_4}{\text{gPHBV}} \right]$, $R = 28 \pm 2.1 \left[\frac{\text{mLCH}_4}{\text{gPHBV} \cdot \text{day}} \right]$, and $\lambda = 5.5 \pm 0.3 [\text{days}]$. There was only a minor decrease (~ 0.5 –1 day) in the value of λ , indicating that the as-collected anaerobic sludge that was used as an inoculum was already well adapted for PHBV degradation. Degradation also initiated rapidly ($\lambda < 1$ day) in the reactors containing the cellulose positive controls with as-collected inoculum, indicating that the inoculum was sufficiently acclimated for cellulosic degradation.

4.2. Methane production and particle size

Fig. 3 illustrates biodegradation of the different PHBV particle size fractions in terms of CH₄ produced and extent of biodegradation. Ultimate CH₄ production is the same for all of the tests

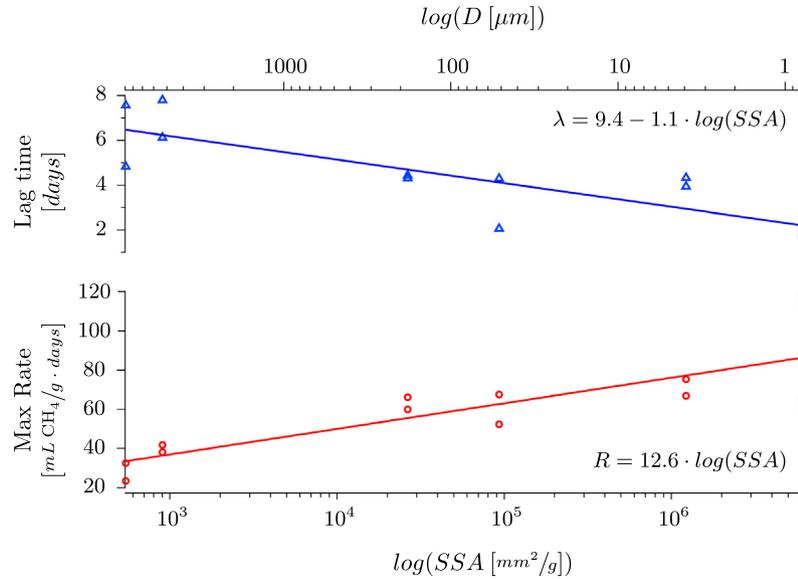


Fig. 5. Plot of maximum rate and lag time versus the log of SSA. Maximum rate and lag time were taken from the derivative of CH₄ production with time (Fig. 3). Fits ($\pm 95\%$ confidence interval) are: (1) $\lambda = A_1 + M_1 \cdot \log(SSA)$, where $A_1 = 9.4 \pm 2.5$ and $M_1 = -1.1 \pm 0.5$ (2) $R = A_2 + M_2 \cdot \log(SSA)$, where $A_2 = 0 \pm 13.1$ and $M_2 = 12.6 \pm 2.7$.

(580 mL/g \pm 12 mL/g) corresponding to a biodegradability of $86 \pm 2\%$, but there is evidence that methanogenesis was temporarily inhibited in particle size fractions below 840 μm . Variability in the CH₄ production response between duplicate samples is potentially due to small differences in particle size profiles added to each bioreactor or reactor-specific variability in the microbial community's ability to convert rapidly generated hydrolysis products to CH₄.

Fig. 4 gives an indication of why gas production initiates, but then lags in the smaller particle size/higher surface area size fractions. Gas composition in the reactor headspace shows increased levels of CO₂ in the initial stages of degradation in the reactors where CH₄ production stalls, Fig. 4a. There is also a corresponding decrease in pH that is more pronounced in the reactors with lower particle sizes, Fig. 4b, due to insufficient buffering capacity of the system. This decrease can be attributed to the build up of VFAs and resultant reactor acidification, which is a known reason for inhibition of CH₄ production in anaerobic systems (Franke-Whittle et al., 2014; Vavilin et al., 2008). With time, the rate of CH₄ generation in all reactors recovered, but this is a clear instance in which hydrolysis rate does not limit the rate of CH₄ production.

In order to evaluate the impact of mechanical pre-treatment, in combination with the kinetic modeling of PHBV degradation, the relationships between lag time, rate and surface area were determined by differentiating CH₄ production over time (Fig. 3). A semi-log plot of SSA yielded a linear relationship with lag time, λ , and maximum rate, R , (Fig. 5). This logarithmic relationship is important, because while rate increases and lag time decreases with increasing surface area, there reaches a point of diminishing returns in single-stage AD where the potential for reactor upsets would outweigh improvements achieved in rate and lag time. Based on the CH₄ production trends, and the pH and gas composition profiles, this point of diminishing return occurs near the upper end of particle sizes (lower end of SSA) investigated in this study. Pommier et al. observed a similar phenomenon in studies of shredding pre-treatment of paper and cardboard (Pommier et al., 2010).

This work suggests that the particle size threshold where the rate of hydrolysis and acetogenesis exceeds that of methanogenesis is close to 0.8 mm. Reduction of particle size below this value would have minimal positive impact on CH₄ generation, with increased potential for reactor upset. While anaerobic communities could likely adapt to smaller particles and become less sensi-

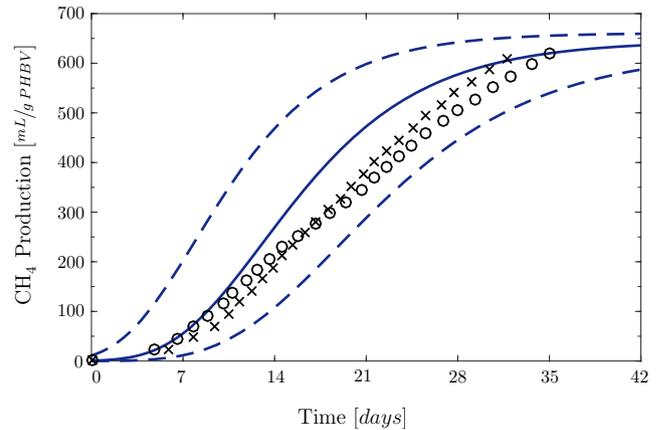


Fig. 6. Prediction (solid line) of CH₄ production from PHBV injection molded samples (with a SA of 544 mm² and a mass of 0.45 g) obtained by combining the Gompertz and surface area model for maximum rate and lag time. The dashed maximum and minimum bands are calculated from the 95% confidence intervals of the fits from Fig. 5 ($R = 35.4 \pm 7.7$ mL/g · day and $\lambda = 6.3 \pm 4.2$ days) and Table 3 ($P_m = 630 \pm 20$ mL/g).

tive to the rapid generation of hydrolysis products at small particle sizes, even continuously operated industrial digesters are sensitive to accumulation of VFAs (Franke-Whittle et al., 2014).

The maximum rate, R and lag time, λ , dependence on SSA from Fig. 5 can be input into the Gompertz model and used in a predictive capacity, as shown in Fig. 6 for injection molded PHBV samples. The maximum and minimum bands in Fig. 6 are calculated from the 95% confidence intervals of the fits from Fig. 5 ($R = 35.4 \pm 7.7$ mL/g · day and $\lambda = 6.3 \pm 4.2$ days) and Table 3 ($P_m = 630 \pm 20$ mL/g). The data for the injection molded samples fall within the combined Gompertz and surface area model prediction.

5. Conclusions

The Monod, Contois, and Gompertz models all provided good fits for CH₄ production from anaerobic biodegradation of the bioplastic PHBV ($R^2 > 0.98$). The Gompertz model proved particularly

useful because it is simple, has parameters with easily interpretable physical meaning, has overall goodness of fit, and can be adapted to enable predictions by incorporating a term for surface area and particle size. A particle size breakpoint for all models was ~0.8 mm. For particles below this size, CO₂ accumulated and pH decreased, indicating that hydrolysis was no longer rate-limiting, and a more complex model is required.

Model derivations, fits, and additional calculations are provided in detail in the supplementary information in [Appendix A](#), along with supporting figures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2016.11.119>.

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