Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations

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Equations to describe gas production profiles, obtained using manual or automated systems for *in vitro* fermentation of ruminant feeds, were derived from first principles by considering a simple three-pool scheme. The pools represented were the potentially degradable and undegradable feed fractions, and accumulated gases. The equations derived and investigated mathematically were the generalized Mitscherlich, generalized Michaelis–Menten, Gompertz, and logistic. They were obtained by allowing the fractional rate of degradation to vary with time. The equations permit the extent of ruminal degradation (hence the supply of microbial protein to the duodenum) to be evaluated, thus linking the gas production technique to animal production.

Rumen: Gas production: Mathematical models

The development of new gas production methods (Theodorou *et al.* 1994) and the automation of existing ones (Beuvink *et al.* 1992) have led to the urgent need for suitable mathematical models to describe and interpret cumulative gas production profiles. These profiles vary in shape from steep diminishing returns to highly sigmoidal, thereby creating a difficulty in the choice of an appropriate model. The slope of the profile tends to be zero in the early stage of fermentation, as there is little or no gas production, giving rise to a lag phase. Thereafter, a steady increase is observed until substrate depletion causes the profile to approach an upper asymptote. Ideally, a function is required which is capable of modelling both a range of shapes with no inflexion point and a range of sigmoidal shapes in which the inflexion point is variable.

Several models, e.g. Mitscherlich, Michaelis–Menten and Gompertz equations, have been proposed for this purpose (Krishnamoorthy *et al.* 1991; Merry *et al.* 1991; France *et al.* 1993; Beuvink & Kogut, 1993; Blümmel & Orskov, 1993; Schofield *et al.* 1994; Groot *et al.* 1996), sometimes blending empiricism with a more mechanistic view based on a compartmental scheme. However, there appears to be a lack of appreciation of the relationship of the various models to each other and to more general models.

Crucially, the models generally fail to establish the link between gas production *in vitro* and extent of degradation in the rumen.

The objectives of the present paper are to present a unifying analysis of this area, pointing out compartmental interpretations of some of the candidate models, and to link the gas production technique to animal performance by deriving an expression for the extent of ruminal degradation for each model. The models are presented under the headings of the generalized Mitscherlich, generalized Michaelis–Menten, and standard growth functions. Mathematically, the approach adopted is to provide a diagram relevant to each model, translate the diagram to mathematics, solve the mathematics, and illustrate the resultant algebraic solution. The models derived herein have been evaluated in a companion paper by fitting them to gas production profiles obtained using a diverse range of ruminant feeds (Dhanoa *et al.* 2000).

Generalized Mitscherlich

Although aspects of the derivation presented in this section have been reported previously (France *et al.* 1993), the

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complete analysis is given here *ab initio* in an attempt to establish a framework for considering the other models.

Consider an amount (g DM) of a particular feed or feed component, such as plant leaf, plant stem or total organic matter (OM), placed in a culture bottle at time zero, and let the component comprise a degradable fraction S and an undegradable fraction U. Degradation occurs at a fractional rate μ (/h) and a discrete lag T (h) is assumed to occur before degradation commences. The three-pool scheme is shown in Fig. 1. The dynamic behaviour of the two feed-component fractions (both pools in g) is described by the differential equations:

$$dS/dt = 0, \quad 0 \leqslant t < T \tag{1a}$$

$$= -\mu S, \quad t \geqslant T, \tag{1b}$$

and

$$dU/dt = 0, \quad t \geqslant 0. \tag{2}$$

In this case the fractional rate of degradation μ is postulated to vary with time t as follows:

$$\mu = b + c/(2\sqrt{t}), \quad t \geqslant T, \tag{3}$$

where b (/h) and c ($h^{-1/2}$) are constants. The conditions $b \geqslant 0$, $c \geqslant -2b\sqrt{T}$ have to be satisfied as μ cannot be negative. Equation 3 permits the fractional degradation rate to remain constant, or to decline or increase asymptotically with time. It is possible to provide a tentative interpretation of equation 3 (France *et al.* 1996). Many diffusion processes exhibit a square root time dependence. For instance, the distance diffused in time t is $\sqrt{(4Dt)}$, where D is the diffusion constant. The diffusion of a growth activator into a growth-producing region, after a lag time, (and by analogy the degradation by microbial enzymes of plant cell-wall material in the rumen) could lead to an activation equation similar to 3.

On substituting for μ using equation 3 and integrating, equations 1 and 2 yield:

$$S = S_0, \quad 0 \leqslant t < T \tag{4a}$$

$$= S_0 e^{-[b(t-T) + c(\sqrt{t} - \sqrt{T})]}, \quad t \geqslant T, \tag{4b}$$

and

$$U = U_0, \quad t \geqslant 0, \tag{5}$$

where S_0 and U_0 are the zero-time quantities of the degradable and undegradable fractions of the feed component respectively.

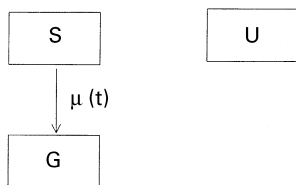If the rate of gas production in the culture bottle is assumed to be directly proportional to the rate of degradation of feed component, then:

$$dG/dt = -YdS/dt, \tag{6}$$

where G (ml) denotes total gas accumulation to time t and Y (ml/g degradable DM) is a constant yield factor. The negative sign is introduced into equation 6 because G and S vary in opposite senses, in that G accumulates as S depletes. Using equation 1b to substitute for dS/dt in equation 6 then differentiating leads to:

$$d^2G/dt^2 = YS(d\mu/dt - \mu^2), \quad t \geqslant T. \tag{7}$$

A point of inflexion exists when d^2G/dt^2 equals zero, i.e.

$$d\mu/dt = \mu^2. \tag{8}$$

Total gas accumulation to time t is given by integrating equation 6:

$$G = Y(S_0 - S). \tag{9}$$

Using equation 4b to substitute for S gives a generalization of the Mitscherlich:

$$G = YS_0\{1 - e^{-[b(t-T) + c(\sqrt{t} - \sqrt{T})]}\}, \quad t \geqslant T. \tag{10}$$

A range of values of c is considered in Fig. 2 which shows the general behaviour of this cumulative gas production equation, whose form can give rise to a variable inflexion point (France *et al.* 1996).

A point of inflexion exists if:

$$-c/(4\sqrt{T^3}) \geqslant [b + c/(2\sqrt{T})]^2 \tag{11}$$

The condition is obtained from equation 8 using equation 3 for μ. This leads to:

$$\sqrt{(-c)} \geqslant \{\sqrt{[1/(2\sqrt{T}) + 4b\sqrt{T}]} - 1/(\sqrt{2}T^{1/4})\}/\sqrt{2}, \tag{12}$$

which, for the parameter values used in Fig. 2, gives $-0.6 \leqslant c \leqslant -0.29$ for an inflexion point. Thus, in Fig. 2, $-0.6 \leqslant c \leqslant -0.29$ gives sigmoidal accumulation, whereas $-0.29 < c < \infty$ gives non-sigmoidal accumulation. The values -0.6 and -0.29 are not invariant, but vary with the choice of the other parameters. The inflexion points for the curves shown in Fig. 2 occur at 22.8 and 23.7 h for c equals -0.6 and $-0.4/h^{-1/2}$ respectively. Equation 12 shows there is no inflexion point for positive c.
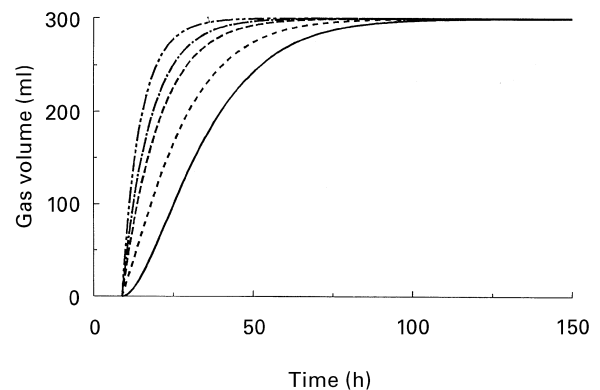

Fig. 1. The three-pool scheme for feed degradation. The pools are: degradable substrate S (g), undegradable substrate U (g) and accumulated gases G (ml). The fractional degradation rate μ (/h) varies over time and a discrete lag is assumed to occur before degradation commences.

Fig. 2. The range of behaviour of the generalized Mitscherlich model with Y = 500 ml/g, S_0 = 0.6 g, b = 0.1/h and T = 9 h. The units of c are $/h^{-1/2}$. (——), c = −0.60; (- - - -), c = −0.40; (— — —), c = −0.10; (− · − ·), c = 0.10; (− · · −), c = 0.50.

We define t_p (h) as the time (from incubation) needed for proportion p of the degradable fraction S_0 to disappear (i.e. be degraded). Equation 4b can be re-arranged to give:

$$bt + c\sqrt{t} - [bT + c\sqrt{T} - \ln(S/S_0)] = 0. \qquad (13)$$

The p-time calculation is obtained from the root of this equation and given by:

$$t_p = [(-c/2 + \sqrt{\{c^2/4 + b[bT + c\sqrt{T} - \ln(1-p)]\}})/b]^2 \qquad (14)$$

for positive b (the other root leads to biologically inadmissible p-time values). The value of t_p will always exceed the lag T. Now p may have any value in the range 0–100 using a percentage scale. For comparison of different feeds based on gas pool size, a value consistent with the recording period is desirable. The time, t_{95}, taken for gas accumulation to reach 95 % of its asymptotic value may provide such an index (say G_{95}) that can be used to rank different substrates. Another useful value is t_{50}, the half-life of the degradable fraction. The half-lives for the curves illustrated in Fig. 2 are 31·7, 23·2, 17·1, 15·1 and 12·9 h for c equal to –0·6, –0·4, –0·1, 0·1 and 0·5/$h^{-1/2}$ respectively. Some workers may prefer to use other values, e.g. t_{25}, t_{75} or the difference $t_{75} - t_{25}$.

In the rumen proper, feed is subjected to passage and thus the dynamics of the two feed-component fractions obey the following differential equations:

$$dS/dt = -kS, \ 0 \leqslant t < T \qquad (15a)$$

$$= -(\mu + k)S, \ t \geqslant T, \qquad (15b)$$

and

$$dU/dt = -kU, \ t \geqslant 0 \qquad (16)$$

where k (/h) is the passage rate constant. On using equation 3 to substitute for μ, their solution is:

$$S = S_0 e^{-kt}, \ 0 \leqslant t < T \qquad (17a)$$

$$= S_0 e^{-[(b+k)(t-T) + c(\sqrt{t} - \sqrt{T}) + kT]}, \ t \geqslant T, \qquad (17b)$$

and

$$U = U_0 e^{-kt}, \ t \geqslant 0. \qquad (18)$$

The extent of degradation in the rumen is given by the equation:

$$E = \int_T^\infty \mu S dt/(S_0 + U_0). \qquad (19)$$

The instantaneous rate of degradation in the rumen at time t (>T) is μS, so the total amount degraded will be the integral over all this time, and the extent of degradation is defined as the total amount degraded divided by the amount entering, which gives equation 19. Using equations 3 and 17b to substitute for μ and S respectively in equation 19, then:

$$E = S_0 I/(S_0 + U_0), \qquad (20)$$

where:

$$I = \int_T^\infty [b + c/(2\sqrt{t})] e^{-[(b+k)(t-T) + c(\sqrt{t} - \sqrt{T}) + kT]} dt. \qquad (21)$$

The integral I is non-analytical (i.e. cannot be integrated to yield an algebraic equation) and has to be evaluated numerically. Obviously, E and I are dimensionless.

Thus, values of S_0 and U_0 (obtained from the gas production technique by measuring the zero-time degradable and undegradable fractions), and estimates of b, c, and T (obtained by fitting equation 10 to the cumulative gas production data), used in conjunction with an estimate of the rate of passage k (obtained using digesta-flow markers and either faecal or ruminal sampling), permit evaluation of the extent of ruminal degradation of a feed or feed component by applying equations 20 and 21 (Dhanoa *et al.* 2000). The yield factor Y can also be obtained from fitting equation 10 given S_0.

Special case

If the fractional degradation rate μ is constant then parameter c is zero, μ equals b, and the gas production equation becomes:

$$G = YS_0[1 - e^{-\mu(t-T)}], \ t \geqslant T. \qquad (22)$$

Equation 22 is an example of the well-known Mitscherlich function which has enjoyed widespread usage amongst ruminant nutritionists in the analysis of *in situ* digestion data (Ørskov & McDonald, 1979). A range of values for μ is shown in Fig. 3 which illustrates the general behaviour of this equation. The rate of accumulation decreases continually and there is no point of inflexion. This can be seen from the second differential (equation 7), which can only be zero when S is exhausted as μ is a positive constant.

For this special case (c = 0, μ = b), the integral I (equation 21) is analytical. Integrating equation 21 gives:

$$E = S_0 e^{-kT} \mu/[(\mu + k)(S_0 + U_0)]. \qquad (23)$$

Thus the extent of degradation in the rumen can now be evaluated using a simple algebraic expression.

Generalized Michaelis–Menten

The scheme is shown in Fig. 1. In this case the fractional degradation rate μ varies with time t as:

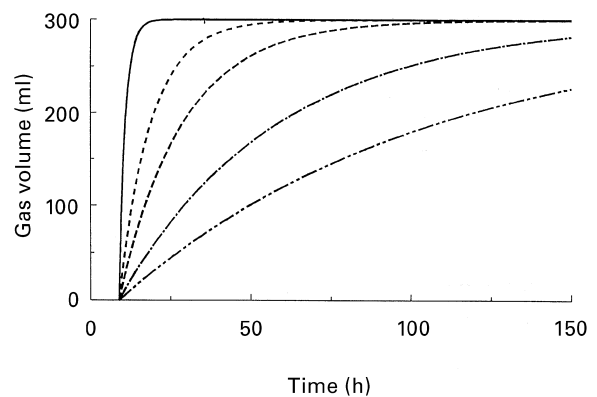$$\mu = c(t-T)^{c-1}/[(t-T)^c + K^c], \ t \geqslant T, \qquad (24)$$

Fig. 3. The range of behaviour of the simple Mitscherlich model with Y = 500 ml/g, S_0 = 0·6 g, and T = 9 h. The units of μ are /h. (——), μ = 0·50; (----), μ = 0·10; (– – –), μ = 0·05; (– · – ·), μ = 0·02; (– ·· – ··), μ = 0·01.

where c (dimensionless) and K (h) are positive constants. The conditions $c > 0$, $K > 0$ have to be satisfied as μ cannot be negative. K is the time after lag time T when half the amount of substrate is degraded (equation 25b). Equation 24 permits the fractional degradation rate to decrease continually ($c \leqslant 1$), or to increase to reach a maximum and then decrease again ($c > 1$).

The dynamics of the two feed-component fractions are given by equations 1 and 2. On substituting for μ using equation 24 and integrating, equations 1 and 2 now yield:

$$S = S_0, \ 0 \leqslant t < T \tag{25a}$$

$$= S_0 K^c / [(t - T)^c + K^c], \ t \geqslant T, \tag{25b}$$

and

$$U = U_0, \ t \geqslant 0. \tag{26}$$

Equation 9 applies, assuming the rate of gas production is directly proportional to the rate of degradation of feed component, giving:

$$G = Y S_0 (t - T)^c / [(t - T)^c + K^c], \ t \geqslant T. \tag{27}$$

A range of values of c is illustrated in Fig. 4 that shows the general behaviour of this equation, which has a variable point of inflexion when $c > 1$.

The point of inflexion satisfies equation 8 which yields the condition:

$$(c - 1)K^c - (c + 1)(t - T)^c = 0. \tag{28}$$

If the point of inflexion occurs at time t* ($>T$), then t* is given by:

$$t^* = T + K[(c - 1)/(c + 1)]^{1/c}, \ c > 1. \tag{29}$$

The inflexion points for the curves shown in Fig. 4 occur at 23·4, 32·1 and 33·5 h for c equals 2, 5 and 10 respectively. The time from incubation needed for proportion p of S_0 to disappear is found using equation 25b and is:

$$t_p = T + K[p/(1 - p)]^{1/c}. \tag{30}$$

The half-lives for the curves in Fig. 4 are all 34·0 h.

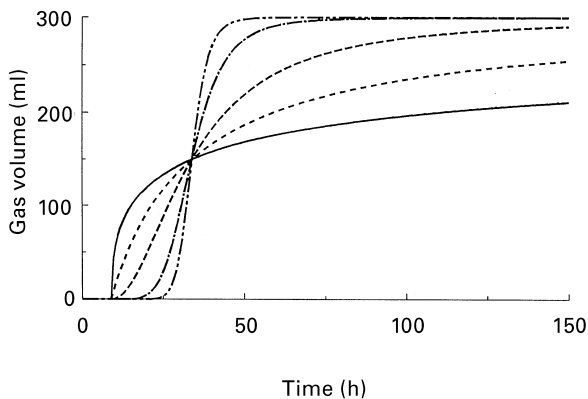In the rumen, where feed is also subject to passage, the two feed-component fractions obey differential equations 15 and 16. Using equation 24 to substitute for μ, their solution is:

$$S = S_0 e^{-kt}, \ 0 \leqslant t < T \tag{31a}$$

$$= S_0 K^c e^{-kt} / [(t - T)^c + K^c], \ t \geqslant T, \tag{31b}$$

and

$$U = U_0 e^{-kt}, \ t \geqslant 0. \tag{32}$$

The extent of degradation in the rumen is given by equations 19 and 20 with:

$$I = cK^c \int_T^\infty \{(t - T)^{c-1} e^{-kt} / [(t - T)^c + K^c]^2\} dt. \tag{33}$$

Again, the integral I is non-analytical and has to be evaluated numerically.

Special case

In this case the parameter c equals 1 so the fractional degradation rate varies with time t as follows:

$$\mu = 1/(t - T + K), \ t \geqslant T, \tag{34}$$

where K (>0) is the inverse of μ_{max}. The gas production equation becomes:

$$G = Y S_0 (t - T)/(t - T + K), \ t \geqslant T. \tag{35}$$

Equation 35 is a rectangular hyperbola and, with T equals zero, is in the form of the well-known Michaelis–Menten equation of enzyme kinetics. A range of values of K is shown in Fig. 5 which illustrates the general behaviour of this equation. The rate of accumulation decreases continually and there is no point of inflexion. This can be seen from the second differential d^2G/dt^2 which is only zero for $t \to \infty$.

The extent of degradation in the rumen is given by equations 19 and 20 with, in this case:

$$I = K \int_T^\infty [e^{-kt} / (t - T + K)^2] dt. \tag{36}$$

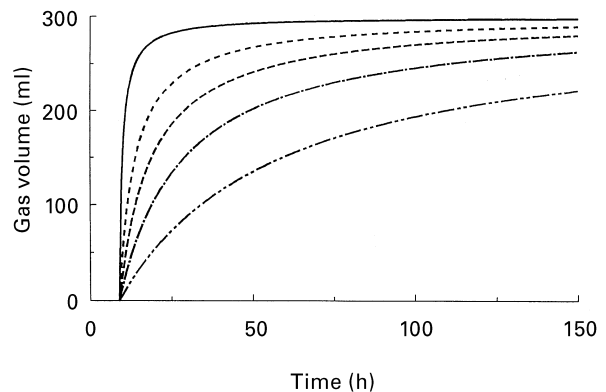This integral is also non-analytical and has to be evaluated numerically.

Fig. 4. The range of behaviour of the generalized Michaelis–Menten model with $Y = 500 \text{ ml/g}$, $S_0 = 0.6 \text{ g}$, $K = 25 \text{ h}$ and $T = 9 \text{ h}$. The parameter c is dimensionless. (——), c = 0·5; (----), c = 1; (– – –), c = 2; (– · –), c = 5; (– · · –), c = 10.

Fig. 5. The range of behaviour of the simple Michaelis–Menten model with $Y = 500 \text{ ml/g}$, $S_0 = 0.6 \text{ g}$ and $T = 9 \text{ h}$. The units of K are h. (——), K = 1; (----), K = 5; (– – –), K = 10; (– · –), K = 20; (– · · –), K = 50.

Standard growth functions

Gompertz

The scheme is shown in Fig. 1. In this case the fractional degradation rate μ varies with time t as:

$$\mu = be^{c(t-T)}, \ t \geqslant T, \tag{37}$$

where b and c (both /h) are constants. The restriction $b > 0$ has to be satisfied as μ cannot be negative and, as explained after equation 38b, the restriction $c \geqslant 0$ also has to be satisfied. Equation 37 permits the fractional degradation rate to remain constant or increase exponentially with time.

Equations 1 and 2 give the dynamics of the two feed-component fractions. On substituting for μ using equation 37 and integrating, these yield:

$$S = S_0, 0 \leqslant t < T \tag{38a}$$

$$= S_0 \exp[-b(e^{c(t-T)} - 1)/c], \ t \geqslant T, \tag{38b}$$

and

$$U = U_0, \ t \geqslant 0. \tag{39}$$

It is evident from an inspection of equation 38b that c ($\geqslant 0$) cannot be negative, because S must be zero at infinite time. Equation 9 applies, assuming direct proportionality between the rate of gas production and the rate of degradation of feed, giving:

$$G = YS_0\{1 - \exp[-b(e^{c(t-T)} - 1)/c]\}, \ t \geqslant T. \tag{40}$$

The behaviour of this cumulative gas equation is illustrated in Fig. 6 using a range of values for c.

A variable point of inflexion exists, provided c is greater than b, and occurs at time:

$$t^* = T + c^{-1}\ln(c/b). \tag{41}$$

The time t* (h) is determined using equation 8, and at this time μ equals c. The t* values for the curves illustrated in Fig. 6 are 41·2, 32·0, 24·0 and 18·2 h for c equals 0·05, 0·10, 0·20 and 0·40/h respectively. The time t_p, that is needed for proportion p of the degradable fraction S_0 to disappear, is found using equation 38b which gives:
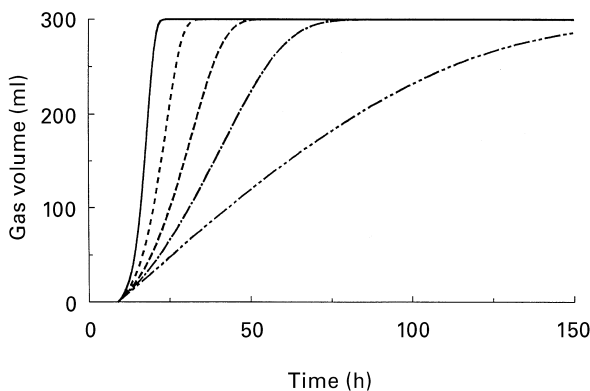
$$t_p = T + c^{-1}\ln\{[b - c\ln(1 - p)]/b\}. \tag{42}$$

Fig. 6. The general behaviour of the Gompertz model with Y = 500 ml/g, $S_0 = 0.6$ g, b = 0.01/h and T = 9 h. The units of c are /h. (——), c = 0.40; (- - - -), c = 0.20; (— — —), c = 0.10; (— · —), c = 0.05; (— · · —), c = 0.01.

The t_{50} values for the curves in Fig. 6 are 61·7, 38·9, 29·7, 22·5 and 17·4 h for c equals 0·01, 0·05, 0·10, 0·20 and 0·40/h respectively.

In the rumen, where feed is also subject to passage, the two feed-component fractions obey differential equations 15 and 16. Using equation 37 to substitute for μ, their solution is:

$$S = S_0 e^{-kt}, \ 0 \leqslant t < T \tag{43a}$$

$$= S_0 \exp[-b(e^{c(t-T)} - 1)/c - kt], \ t \geqslant T, \tag{43b}$$

and

$$U = U_0 e^{-kt}, \ t \geqslant 0. \tag{44}$$

The expressions for extent of ruminal degradation are equations 19 and 20 with:

$$I = b \int_T^\infty \exp[-b(e^{c(t-T)} - 1)/c + (c - k)t - cT]dt. \tag{45}$$

This integral is also non-analytical and has to be evaluated numerically.

Logistic

Again, the scheme is shown in Fig. 1. In this case, the fractional degradation rate μ varies with substrate S rather than explicitly with time:

$$\mu = b(K - S), \ t \geqslant T, \tag{46}$$

where $b > 0$ (g DM/h) and $K \geqslant S_0$ (g DM) are constants. The term $(K - S)$ can be interpreted as a temporal indicator of microbial activity per unit of feed.

The dynamics of the two feed-component fractions are once again given by equations 1 and 2. On substituting for μ using equation 46 and integrating, these equations now yield:

$$S = S_0, \ 0 \leqslant t < T \tag{47a}$$

$$= KS_0/[S_0 + (K - S_0)e^{bK(t-T)}], \ t \geqslant T, \tag{47b}$$

and

$$U = U_0, \ t \geqslant 0. \tag{48}$$

Equation 9 applies, assuming the rate of gas production is directly proportional to the rate of degradation of feed component, giving:

$$G = YS_0\{(1 - e^{-bK(t-T)})$$
$$\div (1 + [S_0/(K - S_0)]e^{-bK(t-T)})\}, \ t \geqslant T. \tag{49}$$

The behaviour of this gas equation is illustrated in Fig. 7 using a range of values for b.

Provided K is less than $2S_0$, a variable point of inflexion occurs when S falls to K/2, i.e. at time:

$$t^* = T + (bK)^{-1}\ln[S_0/(K - S_0)]. \tag{50}$$

The inflexion point occurring at S equals K/2 can be ascertained from equation 8. The t* values for the curves shown in Fig. 7 are 60·2, 34·6, 26·1, 19·2 and 14·1 h for b equals 0·05, 0·10, 0·15, 0·25 and 0·50 g DM/h respectively. The time needed for proportion p of the degradable fraction
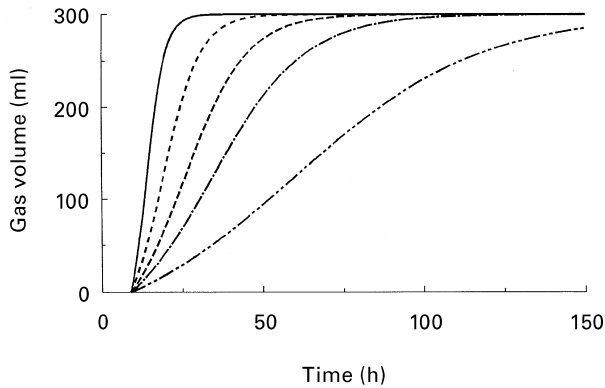
Fig. 7. The general behaviour of the logistic model with Y = 500 ml/g, $S_0 = 0.6$ g, K = 0.07 g and T = 9 h. The units of b are g/h. (——), b = 0.50; (- - - -), b = 0.25; (– – –), b = 0.15; (– · –), b = 0.10; (– - - –), b = 0.05.

S_0 to disappear, t_p, is found from equation 47b:

$$t_p = T + (bK)^{-1}\ln\{[K/(1-p) - S_0]/(K - S_0)\}. \quad (51)$$

The t_{50} values for the curves shown in Fig. 7 are 68·4, 38·7, 28·8, 20·9 and 14·9 h for b equals 0·05, 0·10, 0·15, 0·25 and 0·50 g DM/h respectively.

In the rumen, the two feed-component fractions obey differential equations 15 and 16. Using equation 46 to substitute for μ, their solution is now:

$$S = S_0 e^{-kt}, \quad 0 \leqslant t < T \quad (52a)$$

$$= \theta S_0 e^{-kT}/[S_0 e^{-kT} + (\theta - S_0 e^{-kT})e^{b\theta(t-T)}], \quad t \geqslant T, \quad (52b)$$

and

$$U = U_0 e^{-kt}, \quad t \geqslant 0, \quad (53)$$

where θ denotes $K + k/b$. The expressions for extent of ruminal degradation are equations 19 and 20 with:

$$I = bS_0^{-1}\int_T^\infty (K - S)S\,dt. \quad (54)$$

On replacing S using equation 52b, this integral is non-analytical and has to be evaluated numerically.

Discussion

In the present study, it was decided to construct different models of gas production based on the potentially degradable substrate (S) and its fractional degradation rate (μ). Not only does such a representation allow a proper comparison of the assumptions made in each model, but it also permits the extent of degradation in the rumen (E) (and hence microbial protein supply) to be calculated, thus providing an index of the nutritive value of feeds for animal production. Introducing further complexity into the representation by, for example, adding a microbial pool, would lead to either no analytical solution to the model or an over-parameterized gas production equation. Most published models developed to analyse gas production profiles are based on the amount of gas produced rather than on the amount of substrate degraded, thus failing to link the gas

production technique to animal performance (Krishna-moorthy *et al.* 1991; Beuvink & Kogut, 1993; Blümmel & Ørskov, 1993; Schofield *et al.* 1994; Groot *et al.* 1996).

The simple Mitscherlich function (equation 22) was among the first to be applied to cumulative gas production data (Krisnamoorthy *et al.* 1991; Blümmel & Ørskov, 1993). This equation assumes that degradation occurs at a constant fractional rate μ after a discrete time lag T. Thus, the rate of gas accumulation decreases continually and there is no point of inflexion. In applying the simple Mitscherlich, several research groups have included a parameter α denoting the immediately soluble material when applied to *in sacco* data, or the initial amount of gas produced when applied to gas production data (e.g. Blümmel & Ørskov, 1993; Khazaal *et al.* 1993, 1994). Khazaal *et al.* (1993, 1994) frequently calculated negative values of α, implying a negative initial gas volume. Yet initial gas volumes at the start of incubation should be zero, and in most experimental systems this is achieved by regulating the pressure in the incubation vessels before data are recorded. Thus, the gas production curve starts at zero volume, as in the present representation of the simple Mitscherlich (equation 22). Indeed, this representation of the simple Mitscherlich was applied by Krisnamoorthy *et al.* (1991, 1995).

Following automation of extant systems and development of new ones (Beuvink *et al.* 1992; Pell & Schofield, 1993; Theodorou *et al.* 1994), it became apparent that cumulative gas production profiles varied in shape from steep diminishing returns to highly sigmoidal, and equation forms without a point of inflexion would no longer be appropriate in many cases. France *et al.* (1993) proposed a generalization of the Mitscherlich model (equation 10) to accommodate either sigmoidal or non-sigmoidal shapes. The fractional degradation rate μ can increase or decrease to an asymptote with this equation. This may reflect the increase in substrate accessibility due to hydration of particles, microbial attachment, and increase in microbial numbers after a lag time (McAllister *et al.* 1994). This generalization of the Mitscherlich model has been applied to gas production data from fermentation of roughages with mixed rumen micro-organisms or with single species (Theodorou *et al.* 1994, 1995; Williams *et al.* 1995, 1996; Sileshi *et al.* 1996). However, the assumption that μ approaches an asymptote is not unequivocal. Local build-up of fermentation end-products, reduced availability of essential growth factors, and the imposition of chemical and structural restrictions, are all potential factors which could give rise to a reduced fractional degradation rate as degradation proceeds (Chesson, 1988; Wilson, 1993).

Schofield *et al.* (1994) proposed Gompertz and logistic equations to describe gas production data and compared these with exponential and simple Mitscherlich equations. Their derivations of the Gompertz and logistic equations differ from ours, as they start from the amount of gas produced. In their derivations, the amount of gas produced depends amongst other things on the amount of microbial biomass present. Microbial growth yield is assumed to be constant throughout incubation. Whilst microbial growth yield will be constant in steady-state situations and during the exponential phase in batch cultures (Pirt, 1975), this assumption is unlikely to be valid for the gas production

technique. The Pirt (1975) double reciprocal equation relates efficiency to fractional growth rate of micro-organisms and their non-growth requirements (maintenance), assuming that micro-organisms can utilize substrates for growth only when non-growth requirements have been satisfied. After the inflexion point, the rate of substrate degradation is reduced and microbial non-growth requirements form a larger part of the substrate utilized per unit time, reducing the fractional growth rate and consequently reducing microbial yield. Moreover, microbial growth yield will vary with such factors as microbial species present, pH of the medium, and availability of N substrates (Dijkstra & France 1996), and these factors might change in the course of incubation. In our derivation of the Gompertz and logistic equations (equations 40 and 49 respectively), μ is assumed to increase with time. This increase can be interpreted as an increase in microbial activity per unit feed, but it does not involve any assumptions on the constancy of the microbial growth yield. Schofield et al. (1994) selected the logistic equation to describe gas production curves and applied this equation to describe them for several roughages and for the fibre and non-fibre fractions of roughages (Schofield & Pell, 1995a,b). As with certain applications of the simple Mitscherlich model, an initial gas volume unavoidably arises with application of the Gompertz and logistic equations as given by Schofield et al. (1994), introducing an inherent error as gas volume at zero time should be nil. Note that our Gompertz equation is equivalent to the simple Mitscherlich equation when parameter c equals zero.

Groot et al. (1996) proposed a modified Michaelis–Menten equation without formal derivation to describe gas production curves, and this equation has been applied by Cone et al. (1996). In our derivation of a generalized Michaelis–Menten (equation 27), the fractional degradation rate μ either decreases continuously ($c \leqslant 1$), or increases first and decreases later during fermentation ($c > 1$). Again, an initial increase in μ reflects an increase in substrate accessibility due to hydration of particles, microbial attachment and increase in microbial numbers, whilst the decrease might reflect the imposition of chemical and structural restrictions. Thus, compared with our other gas production models, the assumptions concerning μ are more flexible.

The modified Michaelis–Menten equation has often been extended for multiphasic analysis, assuming that each single phase describes the gas produced by different components of the feedstuff. The number of phases included in a number of applications was two or three (Cone et al. 1996; Groot et al. 1996). Similarly, the logistic and Gompertz equations have been used multiphasically (Schofield et al. 1994; Schofield & Pell, 1995a,b). In general, the robustness of an equation declined when the number of phases was increased, which is an inherent characteristic of these non-linear models. In the present analysis only single-pool characterization of the potentially degradable fraction was considered. For multiple-pool characterization, total gas produced is obtained by simply summing over all the pools of degradable material, so the models derived in the present analysis can be used additively for multiphasic analysis without affecting the basic assumptions inherent in their derivations.

Not all gas production equations have been applied with a fixed lag phase before fermentation starts. Factors which affect the presence and length of a lag include the nature of the substrate incubated, the microbial species inoculated, and the amount of inoculum added (France et al. 1993; Pell & Schofield, 1993; Cone et al. 1996). In fitting the present models the lag phase can simply be deleted from the equation if it does not significantly improve the fit of the curve, except in the case of the generalized Mitscherlich where a lag, albeit small, is needed to prevent a mathematical discontinuity in μ at zero time. There seems little to choose between the present models biologically and mathematically, so choice of best model should be made on the basis of fitting criteria (see Dhanoa et al. 2000).

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