Modelling microbial growth in a closed environment

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Abstract

The formulation of models for the growth of microbes (fungi; bacteria) must not only take account of the current number of the microbes but also of the effect of the environment in which the growth is occurring and of the type of measurements used to record the growth. In industrial processes, the effect of physiological (morphological) changes on the growth must be taken into account. When growth is occurring in an open environment which achieves an equilibrium (e.g. balancing growth and harvesting) and is measured as the time evolution of the total number of microbes present (alive and dead), autonomous ordinary differential equation (ODE) models are appropriate. The corresponding growth measurements (optical density; centrifuged weight), because they record the total number of microbes present (alive and dead), have a logistic structure which autonomous equations, such as the Verhulst, capture. For growth in a closed environment, which is indicative of the situation in laboratory experiments, autonomous ODE models do not necessarily capture the dynamics under investigation. Such situations arise when the question under examination relates to the activity of the surviving microbes, such as in a study of the spoilage and contamination of food, the gene silencing activity of fungi or the production of a chemical compound by bacteria or fungi. Practical and theoretical implications associated with the measurement and modelling of the number of surviving microbes in a closed environment is the focus in this paper. The limitations of current measurement protocols to track the number of surviving microbes are discussed. The use of non-autonomous modifications of autonomous ODE models of growth is proposed and analysed. In particular, a non-autonomous version of the von Bertalanffy model is proposed as an appropriate framework in which to analyse growth in a closed and/or deteriorating environment.

Keywords

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Abstract. The formulation of models for the growth of microbes (fungi; bacteria) must not only take account of the current number of the microbes but also of the effect of the environment in which the growth is occurring and of the type of measurements used to record the growth. In industrial processes, the effect of physiological (morphological) changes on the growth must be taken into account. When growth is occurring in an open environment which achieves an equilibrium (e.g. balancing growth and harvesting) and is measured as the time evolution of the total number of microbes present (alive and dead), autonomous ordinary differential equation (ODE) models are appropriate. The corresponding growth measurements (optical density; centrifuged weight), because they record the total number of microbes present (alive and dead), have a logistic structure which autonomous equations, such as the Verhulst, capture. For growth in a closed environment, which is indicative of the situation in laboratory experiments, autonomous ODE models do not necessarily capture the dynamics under investigation. Such situations arise when the question under examination relates to the activity of the surviving microbes, such as in a study of the spoilage and contamination of food, the gene silencing activity of fungi or the production of a chemical compound by bacteria or fungi. Practical and theoretical implications associated with the measurement and modelling of the number of surviving microbes in a closed environment is the focus in this paper. The limitations of current measurement protocols to track the number of surviving microbes are discussed. The use of non-autonomous modifications of autonomous ODE models of growth is proposed and analysed. In particular, a non-autonomous version of the von Bertalanffy model is proposed as an appropriate framework in which to analyse growth in a closed and/or deteriorating environment.

Keywords. growth modelling, autonomous, non-autonomous, microbial growth, ordinary differential equations, closed environment, food contamination, surviving microbes

1. Introduction

The recovery of information about biological processes often reduces to the modelling of the growth of individuals or populations of individuals. First order ordinary differential equation (ODE) models, utilized for the implementation of the recovery, include (i) the two parameter Verhulst equation [4] and (ii) the four parameter equation proposed by von Bertalanffy [25] in 1957. They are popular because, for a wide range of choices of the parameters, their exact solutions are known and, thereby, the recovery process can be reduced to a curve fitting and regression activity. Their limitations relate to them being autonomous differential equations, which implicitly implies that only the current size of the population controls the growth. The role of the effect of the environment is thereby ignored. Consequently, a corollary of Coleman's [6] 1979 observation that

> "The idea that populations are self-governing systems which regulate their densities in accord with their own properties and those of their environments has long been present in the ecological lit- erature."

is that the modelling of growth is a non-autonomous activity with the role of the environment determining the structure of the non-autonomous terms.

As will be discussed in some detail below, when the matter under investigation relates to knowing the number of surviving microbes, the structure of the corresponding growth curve will, at some appropriate stage, attain a maximum and then decay with the nature of the decay being an indirect measurement of the effect of the environment. However, current measurement protocols, such as optical density and centrifuged weight, only record, indirectly, the number of microbes present (alive and dead). Depending on the situation, the corresponding growth curves will have either a strictly monotone increasing or a logistic (cumulative) structure. It is this fact that underlies the popularity of single first order autonomous ODE models (such as the Verhulst or the von Bertalanffy equation), since the complexity of their solutions is limited to having a monotone increasing, monotone decreasing or logistic structure.

Such solutions are not representative of situations where the growth, after reaching a maximum, decays, which occurs for the number of microbes that survive as the resources available for their survival decrease [17]. The competition leading to death must be taken into account. As stated in Peleg and Corradini [17] (and illustrated in their Figure 1.)

> *"An ideal microbial growth curve is a plot of the number of living cells as a function of time."*

and, thereby, has four basic biological phases – lag; exponential growth; stationary; mortality. Because of the limitations associated with using single autonomous ODEs to model growth, it is necessary to turn to the utilization of non-autonomous and systems of ODEs to model the number of living cells in a closed environment.

The utility and appropriateness of modelling growth as single non-autonomous ODEs are examined in this paper. The focus is non-autonomous versions of the autonomous Verhulst and von Bertalanffy models, along with a discussion of the properties of their solutions. The change from an autonomous to a non-autonomous structure allows the role played by the interaction between the environment and the current size of the population to be included in the modelling.

The possibility of using a coupled system of autonomous ODEs is not pursued here, as this introduces the added complexity about how to model the interaction between the environment and the current size of the population. Experimental protocols for performing the measurement of the number of living cells is not discussed in detail. The interested reader is referred [7, 16, 8].

The paper has been organized in the following manner. Traditional protocols for the measurement of microbial growth and the interpretation of the recorded logistic structure are examined in Section 2. Autonomous models and their solutions are discussed in Section 3 to highlight, in part, the limited structure that their solutions have and their inability to reproduce the four phase growth behaviour of the surviving microbes in a closed or deteriorating environment. Section 4 is devoted to an examination and analysis of non-autonomous models. It includes a discussion of the earlier non-autonomous modelling. The formulation of a simple non-autonomous multiplicative model is used to highlight how the non-autonomous terms control the nature of the growth and the decay. A non-autonomous version of the von Bertalanffy equation is proposed and analysed in Section 5. Conclusions are given in Section 6.

2. Measurements of microbial growth and their interpretation

The traditional protocols for the measurement of microbial growth, in both open and closed environments, include measuring the optical density of the microbes in a representative sub-sample and weight of microbes after the centrifuging of a representative sub-sample. Such a growth curve is an indirect measurement of the actual number of microbes and is usually assumed to be proportional to the number or to be representative of the situation under investigation. The resulting recorded structure, because such measurements include both living and dead microbes, is logistic.

It is this fact that motivates the formulation of autonomous models to explain the growth. However, it is also a bottleneck. The logistic structure hides much of the finer detail of the dynamics such as the proportion of living to dead microbes. There are a number of ways in which the number of either living or dead cells can be measured or estimated [7, 16, 26, 8, 21]. However, they are challenging to implement and time consuming to perform and, therefore, only utilized when circumstances are imperative. Nevertheless, in the study of the population dynamics of microbes, an investigation of when non-autonomous ODE models must be used instead of autonomous ODE ones, represents an important mathematics-for-industry topic from a biological R&D perspective.

This leads naturally to ask the following question:

> *"How should the resulting logistic structure be interpreted?"*.

Not surprisingly, it depends on whether the environment is open or closed. When it can be assumed that an autonomous model is appropriate for the situation and the environment is open, then the model contains a *"harvesting"* term. The prototypical situation is the Verhulst model. The asymptote thereby reflects the fact that the growth is brought into equilibrium by the *"harvesting"* term. For example, in the industrial production of microbes, the harvesting balances off the population reproduction growth which is maintained through the replenishment of nutrients. It is tacitly assumed that there is no need to distinguish between living and dead microbes because the process has been designed to minimize death.

When the environment is closed, if an asymptote occurs, then either all the microbes are dead, because the food supply has been depleted, or, for whatever reason, the living ones have stopped reproducing. As already mentioned, in most closed systems, it is the number of surviving microbes that is required to resolve matters under investigation. This does not negate the utility of the logistic curve, since it contains information like the time it takes for a given type of microbe to fully consume a given amount of some particular class of nutrients. In fact, such interpretations apply more generally in the management of biological organisms. For example, for fish, logistic modelling is important in the design of aquatic farming protocols.

In the deliberations below, the microbial contamination of food is used as the motivation, as extensive literature which includes experimental data is available. An equally important situation arises when the living microbe performs a secondary activity which is under investigation, such as the production of pharmaceuticals and biochemicals or the gene silencing of a transgene by fungi or bacteria. Using microbes such as yeast, fungi or bacteria for the production of secondary metabolites is wide spread and

essential for human health [20].

The big challenge in the study of the population dynamics of the surviving organisms (bacteria, fungi) in a closed environment is the experimental measurement of their numbers (proportion). Some representative experimental measurements of such situations can be found in Figure 13 of Horowitz *et al.* [13] and Figures 3 and 5 in Carvell and Dowd [5]. As already mentioned, most experimental protocols, such as the optical density of well stirred sub-samples [15] and the weight of the solid component after centrifuging, only record (indirectly) the total population (alive and dead). This measurement difficulty is reflected in the fact that a key aspect in Peleg and Corradini [17] is simulating the likely behaviour of the models that they have formulated to reproduce a four phase structure.

3. AUTONOMOUS GROWTH MODELLING

The modelling of biological growth, using autonomous ODE models, has a long history [11, 22, 9].

It appears that the emphasis on autonomous modelling can be traced to a number of independent reasons: in the measurement of the populations of many organisms, it is the evolution of the total population (alive and dead) that is observed; growth and decay processes are popular applications to motivate lectures about autonomous ODEs; growth and decay are both conceptualized as being different rate processes each of which depends only on the current size of the population, which is plausible biologically; biological growth and decay, experimentally and observationally, appears to be a self-limiting process.

The autonomous ODE modelling of the self-limiting growth of a biological population $N(t)$ dates from Verhulst's historic paper of 1838 and takes the form [4]

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right),$$

where $r > 0$, $K > 0$ and t denote, respectively, the growth rate, the carrying capacity (harvesting level) and time. In this simple model, even though the decay is quadratic compared with the linear growth, the solution has a logistic (cumulative) structure. A key generalization of this model is that proposed by von Bertalanffy [25], which takes the form

$$\frac{dN}{dt} = \eta N^m - \kappa N^n, \ \ N = N(t), \ \ t \geq 0, \quad N(0) = N_0, \ (1)$$

with η, m, κ, n and N_0 non-negative. When $n = 1$, equation (1) has a Bernoulli ODE structure, the solution of which, as noted by von Bertalanffy [25], has the explicit form

$$N(t) = \left\{\alpha - (\alpha - N_0^{1-m})\exp\left(-\left(\frac{\eta}{\alpha}\right)(1-m)t\right)\right\}^{1/(1-m)}, \quad (2)$$

where $\alpha = \left(\frac{\eta}{\kappa}\right)$, from which it follows that initial growth or decay is controlled by whether $m > 1$ or $m < 1$. Other analytic solutions are known. For example, when $m = n = 1$,

one obtains the standard exponential growth/decay solution $N(t) = N_0 \exp((\eta - \kappa)t)$, whereas, for $m = n \neq 1$,

$$N(t) = \left[N_0^{1-m} + (1-m)(\eta - \kappa)t\right]^{1/(1-m)}.$$

The solution of equation (1) when $n = 2 - m$, $m \neq 1$, takes the following compact form

$$N(t) = \left[\sqrt{\frac{\eta}{\kappa}}\,\frac{A\exp(2\sqrt{\eta\kappa}(1-m)t) - 1}{A\exp(2\sqrt{\eta\kappa}(1-m)t) + 1}\right]^{1/(1-m)},$$

where

$$A = \frac{\sqrt{\eta} + \sqrt{\kappa}N_0^{1-m}}{\sqrt{\eta} - \sqrt{\kappa}N_0^{1-m}}.$$

For general m and n, the closed form solution for $N(t)$ involves hypergeometric functions, the complexity of which limits their practical utilization.

Even though such models involve a competition between growth and decay, because of their autonomous structure, the complexity of their solutions is limited to monotone increasing, monotone decreasing and logistic (cumulative) behaviour. The reason for this can be easily verified mathematically. Consider the following first order autonomous ODE

$$\frac{dN}{dt} = N'(t) = g(N), \ \ t \geq 0, \ \ N(0) = N_0, \ \ g \in C^1[0, \infty). \tag{3}$$

If $g(N) = 0$ does not have a solution, then $N'(t)$ does not change sign. Consequently, depending on whether $g(N)$ is initially positive or negative, $N(t)$ is correspondingly always increasing or decreasing. Otherwise, let t^* denote the first value of t when $g(N(t^*)) = 0$. It follows immediately from equation (3) that $N'(t^*) = 0$. Differentiation of the autonomous equation (3) with respect to t yields

$$\frac{d^2N}{dt^2} = N''(t) = \frac{dg(N)}{dN}N'(t), \quad t \geq 0. \tag{4}$$

The trajectory of $N(t)$ for $t > t^*$ depends on the sign of

$$N''(t^*) = N''(t)]_{t=t^*} = \left[\frac{dg(N)}{dN}N'(t)\right]_{t=t^*}.$$

However, because $N'(t^*) = 0$, it follows that $N''(t^*) = 0$ which implies that the growth of $N(t)$ asymptotes to the constant value $N(t^*)$. An important form for $g(N)$ is given by $Nf(N) + K$ with K a constant [11], which will be discussed below.

This leads naturally to the conclusion that, for the modelling of the population dynamics of the surviving organisms in a closed environment, in order to accommodate the mentioned four phases involved, the appropriate framework is non-autonomous ODEs. This fact is only acknowledged implicitly in Peleg and Corradini [17] in that the ODEs that they analyse have a non-autonomous structure. In part, this is because their focus is model formulation within a food spoilage context rather than the underpinning mathematics. However, they do comment

"The issue of cell mortality ⋯ is not explicitly addressed by any of the traditional algebraic and rate models".

A quite comprehensive study of the properties of autonomous single variable ODEs can be found in Tsoularis and Wallace [22]. For various choices for the parameters α, β and γ, they examine the properties of the solutions of the following autonomous equation

$$\frac{dN}{dt} = rN^\alpha \left[1 - \left(\frac{N}{K} \right)^\beta \right]^\gamma, \quad t \geq 0, \qquad N(0) = N_0. \quad (5)$$

It has a much more general structure than either the Verhulst or the von Bertalanffy equations, though, with $\gamma = 1$, it can be rearranged to recover the Verhulst and von Bertalanffy forms. The various graphical plots associated with the special cases considered all represent realizations of the point made above about the logistic structure of autonomous single variable ODEs.

4. Non-autonomous growth modelling

As highlighted in the previous section, the conceptualization, which underlies the formulation of autonomous growth models, is the assumption that the growth and the decay are rate processes, each of which depends only on the current size of the population.

From a modelling perspective, how does one highlight the difference between autonomous and non-autonomous modelling?

Implicit in the 1979 quotation of Coleman [6], already cited above, is the conclusion that

"the autonomous structure is appropriate only when the population size alone drives the dynamics", whereas *"the non-autonomous structure is immediately relevant when the environment is assumed to play a role, even if it is minor"*.

4.1. Earlier non-autonomous growth modelling

For different reasons, various non-autonomous versions of the autonomous Verhulst equation have been analysed. For example, the following non-autonomous version of the Verhulst equation, which has been studied by various authors [10, 11],

$$\frac{dN}{dt} = r(t)N \left(1 - \frac{N}{K(t)} \right), \quad r(t), \ K(t) > 0, \quad N(0) = N_0, \quad (6)$$

has the solution [10]

$$N(t) = N_0 \exp \left(\int_0^t r(\tau) \, d\tau \right)$$
$$\times \left[1 + N_0 \int_0^t \frac{r(\tau)}{K(\tau)} \exp \left(\int_0^\tau r(\zeta) \, d\zeta \right) d\tau \right]^{-1}. \quad (7)$$

In a way, the popularity of this non-autonomous form of the Verhulst equation is the fact that its solution has a known analytic form which can be directly exploited. An analysis of the important situation where $r(t)$ and $K(t)$ are slowly varying has been investigated by Coleman [6] and Shepherd and colleagues [10].

Coleman [6] exploits ideas and results from continuum mechanics, related to the concept of "fading memory", to perform his analysis.

Hallam and Clark [11] appear to have been the first to analyse and compare alternative non-autonomous forms of the Verhulst equation for modelling growth in a deteriorating environment. Their paper also contains a detailed discussion as to why the effect of deteriorating environments is an essential feature of real-world population dynamics and thereby cannot be ignored in the associated modelling.

In a detailed analysis of the structure of the solutions of equation (6), Hallam and Clark [11] used an "asymptotic equivalence" analysis to establish that:

(a) For suitably large r, extinction occurs, which is consistent with the situation being modelled.

(b) For suitably small r, the steady state value of the solution exceeds the assumed carrying capacity of the population. Since, biologically, r is interpreted as the growth rate of the population, in the absence of environmental stress, this result leads to the unacceptable conclusion that, for a population which is barely able to persist, it is able to do better in a deteriorating environment.

In order to circumvent this difficulty, Hallam and Clark [11] propose and validate the following model as the more appropriate way to transform the autonomous Verhulst equation to a non-autonomous form for modelling growth in a deteriorating environment

$$\frac{dN}{dt} = N \left(r(t) - c\frac{N}{B(t)} \right), \quad r(t), \ B(t) > 0, \quad N(0) = N_0, \quad (8)$$

where the decay term has been modified to accommodate the change, with c being a measure of the population response to the environmental stress $N(t)/B(t)$ with $B(t)$ denoting the maximum population that the environment can support. This alternative form has the analytic solution

$$N(t) = N_0 \exp \left(\int_0^t r(\tau) \, d\tau \right)$$
$$\times \left[1 + N_0 \int_0^t \frac{c}{B(\tau)} \exp \left(\int_0^\tau r(\zeta) \, d\zeta \right) d\tau \right]. \quad (9)$$

Though equations (7) and (9) have a similar algebraic form, there is a key difference in that the term $r(\tau)/K(\tau)$ in (7) has been replaced by $c/B(\tau)$ in (9). It is the interplay between $r(\tau)$ and $K(\tau)$ in $r(\tau)/K(\tau)$ that is the source of the difficulty highlighted by Hallam and Clarke [11]. In addition, from a modelling perspective, the shortcoming associated with (6) relates to its algebraic form having a

strong coupling of the decay with the growth, which does not arise in (8).

As a consequence of these conclusions, Hallam and Ma [12] studied the following more general form of equation (8)

$$\frac{dN}{dt} = g(N)(r(t) - f(N)N), \quad N = N(t), \qquad (10)$$

for quite general $g(N)$ and $f(N)$. Their goal was to highlight the type of regularity that must be imposed on $g(N)$ and $f(N)$ to guarantee a population dynamics consistent with that associated with a deteriorating environment. The effect of periodic environmental fluctuations for the models (6) and (8) has been examined by Rogovchenko and Rogovchenko [19]. Their results support the conclusions of Hallam and Clark [11] that the model (8) is the more appropriate framework in which to formulate non-autonomous version of the Verhulst equation. In their analysis of persistence and extinction for stochastic non-autonomous logistic systems, Liu and Wang [14] work with a counter part of (8) which has the form (10).

Vance and Coddington [24] and Vance [23], in a study of the extinction and persistence behaviour associated with non-autonomous models of the form

$$\frac{dN}{dt} = Nf(N, t),$$

identified conditions on the time evolution of the environment for which the solution $\bar{N}$ of

$$\frac{d\bar{N}}{dt} = \bar{N}\bar{f}(\bar{N}),$$

yields the essential details about the behaviour of $N(t)$ with $\bar{f}$ corresponding to a long term averaging of $f(N, t)$. In essence, this conceptualizes situations where the population, on average, either continues to increase or asymptote.

Baranyi and colleagues, in a series of papers [2, 3], examined the effectiveness of modelling change in population growth, due to some physiological episodes such as inoculation, in terms of a non-autonomous multiplicative model of the form

$$\frac{dN}{dt} = r(t)f(N)N, \quad N = N(t), \qquad (11)$$

with $r(t)$ chosen to reflect the nature of the episode under investigation. A variety of choices for $r(t)$ are motivated, compared and discussed in some detail in [2, 3].

4.2. MULTIPLICATIVE STRETCHED EXPONENTIAL GROWTH AND DECAY

As suggested by Peleg and Corrandini [17], the competition between growth and decay, because it depends on other factors as well as the current size of the population, could be modelled as a multiplicative interaction between the growth and the decay. Such a model, after an initial growth, allows for a subsequent decrease in the size of the population, as occurs for the survivors in a closed environment. A simple example of such a multiplicative process is

$$N(t) = N_0 \exp\left(\alpha t^\beta\right) \exp\left(-at^b\right), \qquad (12)$$

which models an initial growth (by having $\alpha > a$) which is eventually dominated by the decay (by having $b > \beta$). This multiplicative behaviour is equivalent to the multiplicative process proposed by Peleg and Corradini [17]

$$N(t) = N_0 \exp\left[\left(\frac{t}{t_{cg}}\right)^{m_1}\right] \exp\left[-\left(\frac{t}{t_{cd}}\right)^{m_2}\right] \qquad (13)$$

on setting $m_1 = \beta$, $m_2 = b$, $\alpha = (1/t_{cg})^{m_1}$ and $a = (1/t_{cd})^{m_2}$. Biologically, equation (13) represents the product of interrupted growth from a (positive) initial population with a decay factor with range from zero to one as the population eventually disappears. The parameters t_{cg} and t_{cd} represent the characteristic times of the growth and the decay, respectively, had they been unimpeded. An isothermal chemical kinetics rational for this choice has been given by Peleg *et al.* [18].

This multiplicative growth-decay process for (12) corresponds to the solution of the following non-autonomous ODE

$$\frac{dN}{dt} = \left(\alpha\beta t^{\beta-1} - abt^{b-1}\right) N, \quad N(0) = N_0. \qquad (14)$$

The importance of this structure is that it shows that the four phases can be generated without having to invoke the assumption that the growth and decay rates depend on different powers of the population N. In addition, it shows that the competition between the factors orchestrating the growth and the decay can be modelled and interpreted in terms of non-autonomous terms.

It follows that the value of t^*, at which the right hand side of equation (14) equals zero, is given by

$$t^* = \left(\frac{\alpha\beta}{ab}\right)^{1/(b-\beta)}.$$

Differentiation of equation (14) with respect to t, followed by the replacement of t with t^* and a little algebra yields

$$\frac{b-1}{t^*} > \frac{\beta-1}{t^*} \Rightarrow b > \beta$$

as the condition for

$$\left.\frac{d^2N}{dt^2}\right]_{t=t^*} < 0$$

which guarantees that $N(t)$ has a maximum at $t = t^*$ and that it is the only maximum of $N(t)$.

In their discussion of (13), Peleg and Corradini make some general comments about the behaviour of the terms in their model. Their comments can be formalized and extended as follows:

(i) For $m_1 < 1$, the exponential growth is initially concave upwards and subsequently concave downwards with the cross-over point at

$$t^\# = t_{cg}\left(\frac{1-m_1}{m_1}\right)^{1/m_1}$$

which corresponds to the point where the second derivative of the exponential growth term in equation (13) is zero.

(ii) The $m_1 = 1$ situation corresponds to standard exponential growth which is strictly monotone increasing and is concave upwards.

(iii) For $m_1 > 1$, the exponential growth is only concave upwards as the second derivative of the exponential growth term in equation (13) is always positive.

(iv) Increasing t_{cg} increases the flatness of the "lag" phase before the exponential-like growth becomes apparent.

(v) For $m_2 < 1$, the exponential decay is only concave upwards as the second derivative of the exponential decay term in equation (13) is always positive. In fact, such exponential decay, with $m_2 < 1$, is an example of a completely monotone function [1].

(vi) The $m_2 = 1$ situation corresponds to standard exponential decay which is strictly monotone decreasing and is concave downwards.

(vii) For $m_2 > 1$, the exponential growth is initially concave downward and subsequently concave upward with the cross-over point at

$$t^{\#} = t_{cd} \left(\frac{m_2 - 1}{m_2} \right)^{1/m_2}$$

which corresponds to the point where the second derivative of the exponential decay term in equation (13) is zero.

(viii) Decreasing t_{cg} decreases the time needed for the exponential decay to asymptote to zero, while increasing t_{cd} extends the time taken for the exponential-like decay to disappear.

(ix) When $t_{cg} \approx t_{cd}$ ($t_{cg} < t_{cd}$), the height of the peak is reduced, broadening the width of the "stationary" ("equilibrium") phase.

Interestingly, this multiplicative model reproduces the four phase structure with the evolution of both the growth and decay only interacting with the current size of the population. Some representative plots that are generated by this multiplicative model are shown in Figure 1. The decreasing curves illustrate point (ix) above as t_{cg} approaches t_{cd}.

5. NON-AUTONOMOUS ODE MODELLING

The interesting feature to note about the structure of the right hand side of the ODE (14) is that it separates into an interaction between the current size $N(t)$ of the population and the time evolution of the competition between the growth and decay. It has the same form as the ODE that defines how the funds accumulate in a bank account with
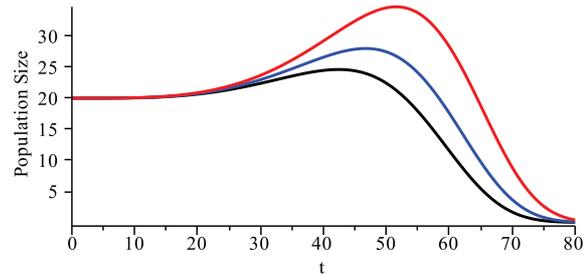

Figure 1: A simulation of the four phase structure using the multiplicative model with $N_0 = 20$, $m_1 = 4$, $m_2 = 5$, $t_{cd} = 44$ and $t_{cg} = 40$ (red), 41 (blue), 42 (black).

the interest rate and the withdrawal strategy taking over the roles of the growth and decay. In addition, this simple non-autonomous model, by taking specific account of the "environment", generates a wider class of solutions than is possible using its autonomous counterpart. However, as noted earlier about the results of Hallam and Clark [11] about the formulation of non-autonomous versions of the autonomous Verhulst model, there is a need to avoid having strong coupling between the time evolution of the growth and decay terms, unless there is compelling evidence otherwise.

In the resulting non-autonomous Verhulst model, the growth interacts with the current size of the population whereas the decay interacts with the square of the current size. On the other hand, the natural generalization of the autonomous of the Verhulst equation to a more general autonomous form, as already noted above, is the von Bertalanffy equation (1). Combining these two observations leads naturally to the non-autonomous form of the von Bertalanffy equation as the framework for the modelling of growth in a closed and/or deteriorating environment

$$\frac{dN}{dt}(t) = \alpha(t)N^{\beta} - a(t)N^{b}(t), \quad N = N(t), \qquad (15)$$

with $\alpha(t)$, β, $a(t)$ and b all non-negative. The multiplicative model (14) is a quite special realization with $\beta = b = 1$.

This thereby yields a quite general non-autonomous structure which allows for general interactions between the current size of the population and the current state of the environment.

5.1. NON-AUTONOMOUS VON BERTALANFFY EQUATIONS

The solutions derived above for the various non-autonomous forms of the Verhulst equation, as well as the multiplicative model, correspond to special cases of the proposed non-autonomous von Bertalanffy equation (15). With β arbitrary and $b = 1$, the Bernoulli ODE is recovered. Applying the transformation $V = N^{(1-\beta)}$ to the

corresponding form of (15) yields the linear equation

$$\frac{dV}{dt} + (1 - \beta)a(t)V = (1 - \beta)\alpha(t).$$

The standard integrating factor approach can then be used to obtain the solution

$$N(t) = \Bigg[\exp\left(-f(t)\right)$$
$$\times \left\{ N_0^{(1-\beta)} - (1 - \beta) \int_0^t \alpha(\tau) \exp\left(f(\tau)\right) d\tau \right\}\Bigg]^{1/(1-\beta)}$$

where $f(t) = (1 - \beta) \int_0^t a(s)\,ds$ and $N(0) = N_0$. When $b = \beta = 1$ the solution

$$N(t) = N_0 \exp\left(\int_0^t \alpha(\tau) - a(\tau)\,d\tau \right)$$

is obtained. The case $b = \beta \neq 1$ has the solution

$$N(t) = \left[N_0^{(1-\beta)} + (1 - \beta) \int_0^t \alpha(\tau) - a(\tau)\,d(\tau) \right]^{1/(1-\beta)}.$$

Setting $\beta = m$, $b = n$, $\alpha(t) = \eta$ and $a(t) = \kappa$ for these solutions recovers the first three solutions to equation (1).

More generally, applying the transformation $V = N^{(1-\beta)}$, $\beta \neq 1$, to (15) yields the nonlinear equation

$$\frac{dV}{dt} = (1 - \beta)\left[\alpha(t) - a(t)V^{(b-\beta)/(1-\beta)}\right].$$

Choosing different values for the power $(b - \beta)/(1 - \beta)$, recognisable first order ODEs are obtained, such as

$$\frac{dV}{dt} = (1 - \beta)\left[\alpha(t) - a(t)V^2\right], \qquad \text{when} \qquad b = 2 - \beta,$$

and

$$\frac{dV}{dt} = (1 - \beta)\left[\alpha(t) - a(t)V^3\right], \qquad \text{when} \qquad b = 3 - 2\beta,$$

which correspond, respectively, to a Riccati and an Abel equation of the first kind.

6. CONCLUSIONS

The growth of microbes in closed environments is a key industrial activity where living microbes perform a secondary activity such as the production of pharmaceuticals and biochemicals. It is equally important in

(a) scientific investigations such as arises in the study of the gene silencing of a transgene by fungi or bacteria,

(b) ecological situations where the environment which sustains life is under challenge (i.e. deteriorating), and

(c) food safety and security where food spoilage and contamination must be identified and controlled.

Because, in such situations, the population dynamics can sometimes have a four phase structure (lag, growth, equilibrium, decay) rather than a logistic structure, the need arises to study the appropriateness or otherwise of autonomous ODE models to explain the observed dynamics. Such a study represents an important *"mathematics-for-industry"* topic from a biological R&D perspective.

Here, after a comparison of autonomous and non-autonomous ODE models in terms of the properties of their solutions, a number of non-autonomous models are formulated and analysed. It is concluded that a non-autonomous version of the von Bertalanffy equation has the appropriate structure and generality to accommodate the modelling of the interaction between current population size and changing environment for the types of biological situations listed above.

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