

# Modeling the Fate of *Escherichia coli* O157:H7 and *Salmonella enterica* in the Agricultural Environment: Current Perspective

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**Abstract:** The significance of fresh vegetable consumption on human nutrition and health is well recognized. Human infections with *Escherichia coli* O157:H7 and *Salmonella enterica* linked to fresh vegetable consumption have become a serious public health problem inflicting a heavy economic burden. The use of contaminated livestock wastes such as manure and manure slurry in crop production is believed to be one of the principal routes of fresh vegetable contamination with *E. coli* O157:H7 and *S. enterica* at preharvest stage because both ruminant and nonruminant livestock are known carriers of *E. coli* O157:H7 and *S. enterica* in the environment. A number of challenge-testing studies have examined the fate of *E. coli* O157:H7 and *S. enterica* in the agricultural environment with the view of designing strategies for controlling vegetable contamination preharvest. In this review, we examined the mathematical modeling approaches that have been used to study the behavior of *E. coli* O157:H7 and *S. enterica* in the manure, manure-amended soil, and in manure-amended soil-plant ecosystem during cultivation of fresh vegetable crops. We focused on how the models have been applied to fit survivor curves, predict survival, and assess the risk of vegetable contamination preharvest. The inadequacies of the current modeling approaches are discussed and suggestions for improvements to enhance the applicability of the models as decision tools to control *E. coli* O157:H7 and *S. enterica* contamination of fresh vegetables during primary production are presented.

**Keywords:** agricultural environment, *Escherichia coli* O157:H7, fate, modeling, *salmonella enterica*

## Introduction

Upon recognition that the use of live stock wastes such as manure and manure slurry in crop cultivation can lead to contamination of fresh produce with human pathogens preharvest, a number of experiments have been carried out to determine the fate of the pathogens in manure, manure-amended soil, and in the manure-amended soil-plant ecosystem (Ongeng and others 2013). Execution of survival experiments whether under controlled environmental conditions or in the field is a fundamental step toward understanding the behavior of manure-borne pathogenic organisms such as *Escherichia coli* O157:H7 and *Salmonella enterica* in the agricultural environment. However, survival studies are often time consuming and provide information limited only to the time period when the experiments were performed. Moreover, when key environmental factors that affect survival change (for example, temperature, moisture, oxygen concentration), then information obtained from previous survival experiments may not be valid and therefore new experiments have to be performed, thus making the approach more expensive over time. Predictive modeling is a potential alternative approach that can overcome the limitations of challenge testing (Baranyi and Roberts 1995; Wilson and

others 2002). This is possible when key environmental factors that affect survival and their respective continuum can be identified and used to develop models that relate these factors to the behavior of the organism under agricultural conditions of interest. In addition, models can enable researchers to make genuine comparisons between treatments so long as parameter values obtained from various treatments according to the model are consistent with practical realities. Furthermore, mathematical models are of value in undertaking quantitative microbiological assessment of vegetables preharvest (Franz and others 2010).

Mathematical models can be categorized into 3 groups: primary, secondary, and tertiary (Whiting and Buchanan 1993). Primary models describe the change of the microbial population as a function of time under particular environmental conditions (McDonald and Sun 1999). The goal is normally to generate information about the microorganism such as generation time, lag phase duration, exponential growth or death rate, and the maximum population density (Whiting and Buchanan 1993, 1994; Whiting 1995). Such information can then be used to compare the effects of experimental variables on the behavior of the microorganism under study. Secondary models describe the response of one or more parameters of a primary model to changes in cultural or environmental conditions of interest (Whiting and Buchanan 1993). Examples of secondary models available in literature include the cardinal parameter models (Augustin and Carlier 2000), square root type models (Ratkowsky and others 1982), and the  $\gamma$  concept models (Zwietering and others 1996). Tertiary models integrate primary and or secondary models into user-friendly software packages. Examples of tertiary models include Growth Predictor & Perfringens Predictor (<http://www.ifr.ac.uk/safety/growthpredictor/>) and Seafood Spoilage and Safety Predictor (SSSP 2009, <http://sssp.dtuqua.dk>).

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Mathematical models can also be categorized as mechanistic or empirical. Mechanistic models are built considering that variables describing the process have biological, chemical, and physical relationships. Empirical models describe the process through a convenient mathematical relationship without considering any intrinsic mechanism occurring during the process (Ross and Dalgaard 2004). Mechanistic models are also known as white-box models, whereas empirical models as black-box models. So far in predictive microbiology, most models are empirical in nature but some of them have been constructed in such a way that biological significance can be adduced from their parameters, such as in the cardinal parameter models (Rosso and other 1995) or in the primary Baranyi model (Baranyi and Roberts 1995), resulting in a hybrid model between the 2 approaches known as gray-box model (Banga and others 2003). Biological significance of parameters is an interesting feature as it guides and eases the parameter estimation process. This was one of the criteria for model inclusion in the GInaFiT software tool that allows users to fit a range of primary models on user-defined data (Geeraerd and others 2005).

Previous reviews that looked at the fate of *E. coli* O157:H7 and *S. enterica* in the agricultural environment focused mainly on survival phenomena without emphasis on the mathematical modeling aspects (Jacobsen and Bech 2011; Akhtar and others 2014; Ongeng and others 2013). In this review, we focused on how primary models have been used to fit survivor curves, and how primary and secondary models were combined to predict survival of *E. coli* O157:H7 and *S. enterica* in the agricultural environment, and to assess the risk of vegetable contamination preharvest. Suggestions on future strategic research direction to enhance the utility of mathematical models as decision tools to control *E. coli* O157:H7 and/or *S. enterica* contamination of fresh vegetables cultivated on manure-amended soils are presented.

### Fitting survivor curves

There are several primary models that can be used to fit data for survival of manure-borne pathogenic bacteria such as *E. coli* O157:H7 and *S. enterica* in manure and/or manure-amended soils. The choice of the model mainly depends on the nature of data. In most cases, several models have to be tried before a final decision can be made based on some criteria, for example, the root mean of squared error (RMSE) and/or coefficient of determination ( $R^2$ ) criteria (Ongeng and others 2011a, b, c, d; Franz and others 2007). The adequacy of the model fit can also be evaluated by looking at the confidence interval and predicted interval of the fitted data points (Ongeng and others 2011a, b, d). This can be achieved by making use of the lsqnonlin procedure of the MatLab Optimization Toolbox ([www.mathworks.com](http://www.mathworks.com)) or any other appropriate numerical tool. In lsqnonlin procedure, the least squares method is used to estimate the parameters of nonlinear equations. The aim is to minimize the sum of squares of the differences between the predicted (model line) and observed values (data points).

The classical log-linear model (Eq. (1)) is the simplest and most straightforward primary model that has been used to fit linear survivor curves of *E. coli* O157:H7 and/or *S. enterica* in manure and/or manure-amended soil (Bolton and others 1999; You and others 2006; Ongeng and others 2011a, b).

$$\log N(t) = \log N_0 - \frac{k_{\max} \times t}{\ln(10)} \quad (1)$$

In Eq. (1),  $N(t)$  is cell density (CFU/g) at time  $t$  (days),  $N_0$  is the initial cell density (CFU/g), and  $k_{\max}$  is the first-order inactivation rate constant ( $\text{day}^{-1}$ ). The log-linear model was originally developed to fit data obtained from isothermal experiments in thermo-bacteriology (Bigelow and Esty 1920). The underlying assumptions governing the log-linear model are that thermal resistances of cells within a population is homogeneous (Anderson and others 1996) and that the probability of inactivation of an organism at a particular time following thermal stress is independent of the exposure time (Peleg 1998). In principle therefore, this model would only be suitable for fitting data obtained from survival experiments performed under isothermal conditions (You and others 2006; Ongeng and others 2011b). However, the model has also been successfully used to fit data obtained from survival studies performed under fluctuating temperature conditions in agricultural fields (Bolton and others 1999; Ongeng and others 2011b). This suggests that the inactivation of the target pathogens as observed in those studies was not primarily due to thermal stress but rather due to other nonthermal factors (for example, biological interaction), which were not influenced by temperature under experimental conditions in which the respective studies were carried out.

In most situations, survivor curves of *E. coli* O157:H7 and *S. enterica* in manure/manure-amended soils exhibited curvilinear shapes. In such situations, a linear model proved inappropriate and therefore nonlinear models had to be used to fit such data sets (Franz and others 2005; Semenov and others 2007, 2009; Ongeng and others 2011a, c, d). On a theoretical basis, the nonlinear behavior of survivor curves can be viewed as the cumulative distribution of an underlying distribution of the decline kinetics (Franz and others 2007). This theory parallels the vitalistic concept that presupposes the emergence of phenotypic variability within a genetically homogeneous population when subjected to stress (Skandamis and others 2002). In general, nonlinearity of survivor curves can therefore be taken to reflect the inactivation phenomena of a population of cells that is heterogeneous with respect to the decline kinetics (Van Boekel 2002; Peleg 2003). This is albeit the unresolved contention regarding the sole use of survivor curves to denote whether or not phenotypic variability exists in a given population (Peleg and others 2005; Stone and others 2009). In depth discussions on fundamentals of the log-linear deviations are beyond the scope of this work. We only focus on how survival data exhibiting curvilinear behavior have been fitted.

The Weibull function (Eq. (2)) is a simple model that can be fitted to the data exhibiting simple nonlinear deviations such as the concave and convex shapes (Stone and others 2009). The utility of the Weibull function in survival studies is based on the premise that the resistance of the test organism to stress as encountered in the survival matrices follows a Weibull distribution. In addition, the survival curve as defined by the Weibull model is taken as a cumulative form of the Weibull distribution of individual inactivation kinetics (Mafart and others 2002; van Boekel 2002).

$$\log N(t) = \log N_0 - \left(\frac{t}{\delta}\right)^p \quad (2)$$

In Eq. (2), all parameters are as defined in Eq. (1) except  $p$ , which denotes shape parameter and  $\delta$ , which indicates time for the first decimal reduction of the original cell count. In practical application, when  $p > 1$  then the shape is convex, when  $p < 1$  the shape is concave, and when  $p = 1$  then the Weibull model assumes the status of a log-linear function. This model was used

to compare survival capabilities of *E. coli* O157 in manure between human and animal isolates (Franz and others 2011) and to study the effect of redox potential on survival of *E. coli* O157:H7 and *S. Typhimurium* in cattle manure and manure slurry (Semenov and others 2011). From the work of Semenov and others (2011), it is quite interesting to note that although curves for data obtained from aerobic experiment were concave in nature, curves for data obtained from anaerobic experiments exhibited convex curvature instead thus indicating greater resistance to inactivation under anaerobic than under aerobic condition.

When survival data suggest a more complicated deviation from linearity, then the capability of the simple Weibull model becomes limited. A typical such nonlinear behavior of survivor curves was observed in experiments where, upon introduction of the test organisms into the survival matrix, the microbial cell number first remained unchanged for a few days, after which the cell population declined linearly (Ongeng and others 2011c). The log-linear shoulder function described by Eq. (3) (Geeraerd and others 2000) seems appropriate for fitting such datasets because the “shoulder length” parameter of the model can account for the apparent initial delay in inactivation.

$$\log N(t) = \log N_0 \times e^{-k_{\max} \times t} \times \frac{k_{\max} \times s_l}{1 + (e^{k_{\max} \times s_l} - 1) \times e^{-k_{\max} \times t}} \quad (3)$$

In Eq. (3),  $N(t)$ ,  $N_0$ , and  $k_{\max}$  are as defined in Eq. (1).  $s_l$  is the shoulder length (days). This model was successfully used to study the effect of cabbage plant rhizosphere on survival of *E. coli* O157:H7 and *S. Typhimurium* in manure-amended soil under tropical field conditions in the Central Agro-Ecological Zone of Uganda (Ongeng and others 2011d).

Survivor curves may, however, exhibit a tailing phenomenon when cell counts are still above the detection limit of the enumeration technique. The Biphasic model with shoulder depicted by Eq. (4) (Geeraerd and others 2005) can adequately describe such a survivor curve on the basis of the assumption that the microbial population in question is heterogeneous and composed of 2 distinct subpopulations that decay independently following the log-linear kinetics (as in Cerf 1977).

$$\begin{aligned} \log N(t) = & \log N_0 + \log \left[ f \times e^{-k_{\max 1} \times t} \right. \\ & \times \frac{k_{\max 1} \times s_l}{1 + [e^{k_{\max 1} - 1}] \times e^{-k_{\max 1} \times t}} + [1 - f] \times e^{-k_{\max 2} \times t} \\ & \left. \times \left[ \frac{e^{k_{\max 1} \times s_l}}{1 + (e^{k_{\max 1} \times s_l} - 1) \times e^{-k_{\max 1} \times t}} \right] \frac{k_{\max 2}}{k_{\max 1}} \right] \quad (4) \end{aligned}$$

In Eq. (4),  $N(t)$ ,  $N_0$ , and  $t$  are as defined in Eq. (1),  $f$  is the fraction of the initial population in a major less-resistant subpopulation,  $(1 - f)$  is the fraction of the initial population in a minor more-resistant subpopulation (being minor at  $t_0$ ),  $k_{\max 1}$  and  $k_{\max 2}$  ( $\text{day}^{-1}$ ) are the specific inactivation rates of the 2 subpopulations, and  $s_l$  is the initial shoulder length (days). This model was successfully applied to study the survival of *E. coli* O157:H7 and *S. Typhimurium* in manure held at 4 °C (Franz and others 2007). As is the case with the log-linear shoulder model, the biphasic model also takes into account the initial delay in decline of cell population by the shoulder length parameter ( $s_l$ ). Although the biphasic model assumes the existence of 2 distinct subpopulations, each with its own inactivation characteristics, Franz and others (2007) suggested an alternative interpretation when they applied

the model to describe survival of *E. coli* O157:H7 in cattle manure. The authors proposed that the observed biphasic pattern of the survivor curves was due to the changing competition pressure resulting from the changing nutrient status of the manure substrate over time, but not due to the existence of 2 distinct subpopulations *per se*. It would therefore be interesting to validate the proposed interpretation using a well-designed experiment that provides data on nutrient dynamics and population dynamics of the background microbial community during survival of the target pathogen.

Pathogen decline curves can sometimes follow a sigmoid-like pattern. This has been observed in some experiments performed under fluctuating environmental conditions as well as in other studies conducted under controlled environmental set ups in the laboratory. In the case of experiments performed in the field, the Double Weibull model provided the best-fit for the data (Ongeng and others 2011a, b, d). This function is defined by Eq. (5) (Coroller and others 2006).

$$\log N(t) = \log \left( \frac{\log N_0}{1 + 10^\alpha} \left[ 10^{-\left(\frac{t}{\delta_1}\right)^p + \alpha} + 10^{-\left(\frac{t}{\delta_2}\right)^p} \right] \right) \quad (5)$$

In Eq. (5):  $N(t)$ ,  $N_0$ , and  $t$  are as defined in Eq. (1),  $p$  is the shape parameter (dimensionless),  $\delta_1$  is the time for the first decimal reduction of subpopulation 1 (days),  $\delta_2$  is the time for first decimal reduction of the second subpopulation (days), and  $\alpha$  is the  $\log_{10}$  of the ratio of the fraction of more sensitive subpopulation to the fraction of less sensitive subpopulation at time zero. The central tenet of the Double Weibull model is that, the function partitions the overall population of the test organism into 2 subpopulations 1 and 2 based on the assumption that subpopulation 1 is more sensitive to the environmental stress than subpopulation 2. Indeed, plots of the Double Weibull model published in Ongeng and others (2011a) seem to indicate the 2 fractions with the first and second fraction exhibiting a concave shape upward and downward, respectively.

The sigmoid-like survivor curves have also been fitted using a modified logistic regression model (Franz and others 2005; Semenov and others 2007, 2009). This modified logistic function, as inspired by the seminal work of Zwietering and others (1990) in microbial growth modeling in foods, is denoted by Eq. (6) according to the formulation of Semenov and others (2007).

$$\log N(t) = \frac{a}{1 + c \times \exp^{-m \times t}} \quad (6)$$

In Eq. (6),  $N(t)$  is cell density (CFU/g) at time  $t$  (days),  $a$  is the upper asymptote (CFU/g),  $c$  is a parameter for the shoulder (days), and  $m$  is a slope parameter for the decline rate ( $\text{days}^{-1}$ ). The appropriateness of the modified logistic model for fitting sigmoid-like survivor curves seems to emanate from the fact that a real sigmoid function is just a special case of the general logistic equation. Using this model, Franz and others (2005) compared data for survival of *E. coli* O157:H7 in manure-amended soil between conventionally managed and organically managed soils and showed that *E. coli* O157:H7 survived longer in manure-amended soil with soil samples from the former than from the latter management system. In addition, Semenov and others (2009) fitted survival data for *E. coli* O157:H7 and *S. Typhimurium* using the same model and showed based on model parameters that surface application of manure to soil poses less risk of plant contamination compared to injection of manure slurry into the soil. In all these situations,

the results according to model parameters were consistent with practical realities.

### Predicting survival and risk assessment of vegetable contamination preharvest

In depth scrutiny of literature reveals that very little work has been done on the development and/or application of mathematical models to predict survival of human enteric pathogenic bacteria such as *E. coli* O157:H7 and *S. enterica* in the agricultural environment. The only known publications available to date are those of Semenov and others (2010) and Ongeng and others (2011d). Semenov and others (2010) modified and extended the BACWAVE model of Zelenev and others (2000) into a COLIWAVE simulation model to predict the survival of *E. coli* O157:H7 in manure and manure-amended soil under dynamic environmental conditions. The COLIWAVE model consists of 3 ordinary differential equations depicting dynamics in the biomass of *E. coli* O157:H7, the biomass of copiotrophic bacteria, and substrate concentration. The model predicts the oscillatory behavior and survival of *Escherichia coli* O157:H7 in manure and manure-amended soil matrices by integrating the relative effects of temperature, oxygen concentration, substrate content, and intra- and inter-specific competition by autochthonous microbial community according to a Lotka–Volterra term. Because of the large number of terms in the model, we have preferred not to present the model here, but refer readers to the original publication by the authors (Semenov and others 2010). The model can be considered semimechanistic (as also denoted by McMeekin and others 2010) as it combines the 3 ordinary differential equations (primary model) containing terms corresponding to existing, mechanistic knowledge about microbial survival, whereas the relations between the primary model parameters (such as the maximum relative growth rate and the intra- and inter-specific competition parameters) and temperature and oxygen concentrations have an empirical (black-box) nature. Simulation trials with the model indicated that the overall decline of *E. coli* O157:H7 counts in cattle manure was primarily determined by competition with autochthonous copiotrophic microbial community, whereas the relative effects of changes in temperature on simulated survival time were more pronounced than changes in oxygen condition. A user-friendly version of the COLIWAVE model based on the Runge–Kutta integration method is available for use in MATLAB ([www.mathworks.com](http://www.mathworks.com)) and can be obtained from the authors on request (Semenov and others 2010).

In the work of Ongeng and others (2011d), a kinetic-based modeling approach to predict the survival of manure-borne enteric pathogenic bacteria in manure and manure-amended soil in agricultural field conditions was proposed. The modeling approach involves the recording of the temperature profile that the organism experiences in the field matrix followed by establishing the survival kinetics under isothermal conditions at a range of temperatures registered in the matrix in the field and then using the isothermal-based kinetic models to develop models for predicting survival under dynamic conditions. The working hypothesis of the proposed modeling approach is that the inactivation phenomena associated with the survival kinetics of an organism in an agricultural matrix under dynamic temperature conditions to be largely attributed to the cumulative effect of inactivation at various temperatures within the continuum registered in the matrix in the field. Following this modeling approach, the authors developed a model which predicted survival time of *S. Typhimurium* in manure and manure-amended soil under tropical field conditions in the

Central Agro-Ecological Zone of Uganda by up to 84% and 71%, respectively (Eq. (7)).

$$\frac{dM^*}{dt} = v_1 + v_2 - v_3 - v_4 \quad (7)$$

In Eq. (7),  $N_t$  is the population at any time instant (CFU/g),  $N_p$  is the population at a previous time instant (CFU/g),  $k_{\max}$  is the decline rate ( $\text{day}^{-1}$ ) at temperature  $T$  corresponding with the time instant  $N_p$  is present, and  $t_d$  is the elapsed time between 2 temperature measurements.

An essential step in predictive model development and application is the validation process. The objective is to ensure that predictions made by the model are useful in real situations. Validation process encompasses comparing predictions of the model with independent data, that is, data different from those used to build the original model (Brocklehurst 2004). Models cannot be used with confidence to make predictions until the validation step is successfully accomplished (Whiting 1995). However, model validation process is often limited by costs of gathering new data from real situations, thus a common approach is the use of previously published data. In the case of the COLIWAVE model (Semenov and others 2010), validation with previously published data (Kudva and others 1998; Fremaux and others 2007; Franz and others 2005, 2008) showed that anaerobic condition was conducive for survival of *E. coli* O157:H7 in manure, a fact which was later confirmed in a well-controlled experiment undertaken by Semenov and others (2011). The kinetic-based model of Ongeng and others (2011d) was also validated using the same approach. The authors used data from other field survival experiments conducted with *S. Typhimurium* at inoculum level of 4 log CFU/g (Ongeng and others 2011b, 2011c) and showed that cultivation of cabbage on contaminated manure-amended soil 61 to 68 days post manure amendment to soil would present less risk of vegetable contamination with the pathogen at harvest.

### Limitations of the currently applied mathematical models and suggestions for future strategic direction

There are 3 important limitations associated with the application of primary models described by Eq. (1) to (6) in fitting survival data. First, most of these models are empirical or, at best, semimechanistic and as such they cannot adequately account for the phenomena that determine survival thus providing very little information on the behavior of the pathogens in the agricultural environment. Despite this drawback, it is important to appreciate that a fully mechanistic model that incorporates all factors that affect survival (for example, physical and chemical characteristics of manure and soil; weather or atmospheric conditions, biological interactions, agricultural and livestock management practices, strain type) may be difficult to develop considering the fact that several factors interact to determine survival (Ongeng and others 2011a, 2013). This dilemma is not peculiar to this situation only, it has also been observed in predictive food microbiology (Mejlholm and others 2010).

Second, the primary models depicted by Eq. (1) to (6) do not take into account oscillations (due to growth/regrowth phenomenon) in the population of test organisms as is often observed in survival data sets (Vidovic and others 2007; Semenov and others 2008; Ongeng and others 2011a), thus failing to accurately describe actual behavior of the pathogens in the agricultural environment. The “quasi-chemical” kinetics model developed by Taub and others (2003) to describe the growth and death of

*Staphylococcus aureus* in intermediate moisture bread might provide a solution to this dilemma because the model takes into account both the death and growth kinetics of the organism under study. The model consists of a set of 4 ordinary differential equations (Eq. (8) to (11)) that integrates the concepts of chemical kinetics and predictive microbiology. The 4 equations describe a scheme representing the microbial life cycle (lag, exponential/growth, stationary, and death phase) in an analogous form to chemical reaction mechanisms.

$$\frac{dM}{dt} = -v_1 \quad (8)$$

$$\frac{dM^*}{dt} = v_1 + v_2 - v_3 - v_4 \quad (9)$$

$$\frac{dA}{dt} = v_2 - v_3 \quad (10)$$

$$\frac{dD}{dt} = v_3 + v_4 \quad (11)$$

where  $M$  represents the concentration of cells in the lag phase,  $M^*$  represents the concentration of cells in the growth phase,  $A$  is the antagonistic metabolite that interacts with multiplying cells,  $D$  denotes the dead cells, and  $v$  being the velocity of the reaction during activation ( $v_1$ ), multiplication ( $v_2$ ), sensitization ( $v_3$ ), and death ( $v_4$ ) of cells. This equation set (Eq. (8) to (11)) describes processes that correspond to the formation and/or elimination of the entities  $M$ ,  $M^*$ ,  $A$ , and  $D$ . The theoretical basis governing the model is well described by Taub and others (2003) and expounded in a mathematical manner by Ross and others (2005). An additional advantage offered by this model is that it can fit both the linear and curvilinear inactivation plots as illustrated with generic *E. coli* in model food systems (Doona and others 2005).

Third, the primary models represented by Eq. (1) to (6) are characteristically static in nature. This means that those models were only appropriately used in experiments conducted under static environmental conditions (Franz and others 2005, 2011; Semenov and others 2011; Ongeng and others 2011a) but were inappropriately applied in studies conducted under dynamic environmental conditions (Bolton and others 1999; You and others 2006; Ongeng and others 2011b, c, d). This is particularly important considering the fact that survival time of *E. coli* O157:H7 in cattle manure was found to be significantly shorter under dynamic temperature conditions than under static temperature conditions (Semenov and others 2007). The modified Weibullian-log logistic (WeLL) inactivation model (Eq. (12); Corradini and Peleg 2008) could be a feasible option for fitting nonisothermal data because of the presence of a logistic adaptation factor that makes the inactivation rate parameter of the WeLL model a function of both temperature and exposure time.

$$\frac{d\log_{10} S(t)}{dt} = -\ln \left\{ 1 + \exp \left\{ K [T(t) - T_c] \right\} \right\} \times n \times \left[ \frac{\log_{10} S(t)}{\ln \left\{ 1 + \exp \left\{ k [T(t) - T_c] \right\} \right\}} \right]^{\frac{n-1}{n}} \quad (12)$$

where  $S(t)$  is the instantaneous survivor ratio, that is  $N(t)/N_0$ ,  $n$  is a temperature-dependent constant (equivalent to the  $p$  shape parameter of the Weibull model),  $T_c$  is the temperature level of inactivation onset,  $T$  is the instantaneous temperature,  $t$  is the time,

$K$  is the temperature dependence of the slope of the inactivation rate parameter. Theoretical background on the manipulation of the original WeLL model to the modified version depicted in Eq. (12) is presented by the authors. Future studies could therefore envisage using this model taking into account the possibility of adjusting the model to include other factors that affect survival in addition to temperature.

The prediction models of Semenov and others (2010) and Ongeng and others (2011d) presented above provide opportunity for the application of mathematical models in risk assessment of fresh vegetables preharvest. However, there are a number of limitations that need to be addressed. The kinetic-based model (Ongeng and others 2011d) was derived based on a single bacterial strain, one soil type, and using inocula at the exponential phase. The model needs to be validated with various soil types and diverse pathogen strains. This is because survival of *E. coli* O157:H7 and/or *S. enterica* in agricultural matrices has been shown to be affected by strain origin (Franz and others 2011) and soil management system (Franz and others 2005). Second, the model was developed using only field temperature data obtained from tropical conditions in sub-Saharan Africa and as such cannot be applied in other geographical regions with different temperature regimes, thus the need for improvement. In addition, performance of the model was 61% to 68% indicating that, notwithstanding experimental errors, the model requires improvement through incorporation of other factors that affect survival.

The COLIWAVE model (Semenov and others 2010) was derived based on assumptions which may not be valid in reality. For instance, the authors considered that *E. coli* O157:H7 population in sterilized and fresh manure would have the same physiological characteristics, that is, the basic relative growth and death rate would be the same. However, as also indicated by the authors, in fresh manure, *E. coli* O157:H7 population would have a different death rate due to competition with the autochthonous microbial community. Another limitation of the COLIWAVE model is the empirical nature of the Lotka-Volterra terms used to describe the inter- and intra-specific microbial competition. The model could be improved by replacing the empirical Lotka-Volterra terms for competition by more mechanistic components (for example, specific metabolites). In addition, the model lacks stochastic effects. It can be expected that both the initial level of pathogens and autochthonous bacteria are in reality, not deterministic values. Furthermore, environmental conditions are also not constant throughout space in manure and manure-amended soil, which would necessitate the development of differential equations which are not only time dependent but also place dependent. Alternatively, following a defined scheme as in microbiological risk assessment, one may envisage a Monte Carlo simulation of the processes taking place in the manure or manure-amended soil, giving answers to questions related to the chance of a pathogen population being able or not to survive a certain environmental condition during a certain amount of time. For example, if it were possible to characterize the statistical distribution (related to biological variability) of some of the 31 input parameters of the COLIWAVE model, a Monte Carlo simulation would lead to confidence bands surrounding the microbial evolution (as in Ongeng and others 2011d) and it would also lead to an assessment of the final risk incorporating biological variability. More details on the assumptions underlying the COLIWAVE model and their limitations thereof in the utility of the model to predict survival were ably discussed by the authors and shall not be presented here any further.

## Conclusions

This review has revealed that application of mathematical models to study the behavior of *E. coli* O157:H7 and *S. enterica* in manure, manure-amended soil and manure-amended soil-plant ecosystems has mostly been directed toward fitting survivor curves of the pathogens, whereas work on the application of the models to predict pathogen survival and to assess the risk of vegetable contamination preharvest has been rather limited. The curve fitting models applied so far are static in nature, mostly nonmechanistic or at best semimechanistic and do not consider fluctuations in cell counts as affected by pathogen regrowth phenomenon. To enhance the utility of models as part of the decision tools in the management of preharvest safety of vegetables, more work needs to be done to develop models that are dynamic, mechanistic, and directed toward prediction and assessment of vegetable safety during cultivation rather than just curve fitting, and we recommend the COLIWAVE model as an elegant model example in this respect that should be improved. Translation of concepts of microbiological risk assessment normally applied in food products to assess risks of fresh vegetable contamination in the field may also be an interesting way of applying the principle of biological variability to find out the most influential risk management options.

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## Author Contributions

D. Ongeng conceptualized the review article, collected initial literature, and drafted the manuscript. L. Haberbeck, G. Mauriello, J. Ryckeboer, D. Springael, and A. Geeraerd collected additional literature and revised the manuscript.

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