Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Kinetic model-based prediction of the persistence of *Salmonella enterica* serovar Typhimurium under tropical agricultural field conditions

D. Ongeng[1,2], C. Muyanja[3], J. Ryckeboer[2], D. Springael[2] and A.H. Geeraerd[4]

1 Department of Food Science and Post Harvest Technology, Faculty of Agriculture and Environment, Gulu University, Gulu, Uganda
2 Division of Soil and Water Management, Department of Earth and Environmental Sciences, Faculty of Bioscience Engineering, Katholieke Universiteit Leuven, Leuven, Belgium
3 Department of Food Science and Technology, Makerere University, Kampala, Uganda
4 Division of Mechatronics, Biostatistics and Sensors (MeBioS), Department of Biosystems (BIOSYST), Faculty of Bioscience Engineering, Katholieke Universiteit Leuven, Leuven, Belgium

Keywords
agricultural environment, isothermal data, kinetic model-based, prediction, survival.

Correspondence
Duncan Ongeng, Department of Food Science and Post Harvest Technology, Faculty of Agriculture and Environment, Gulu University, PO Box 166 Gulu, Uganda.
E-mail: duncanongeng@hotmail.com

2010/2031: received 10 November 2010, revised 10 January 2011 and accepted 17 January 2011

doi:10.1111/j.1365-2672.2011.04958.x

Abstract

Aim: Present a kinetic model-based approach for using isothermal data to predict the survival of manure-borne enteric bacteria under dynamic conditions in an agricultural environment.

Methods and Results: A model to predict the survival of *Salmonella enterica* serovar Typhimurium under dynamic temperature conditions in soil in the field was developed. The working hypothesis was that the inactivation phenomena associated with the survival kinetics of an organism in an agricultural matrix under dynamic temperature conditions is for a large part due to the cumulative effect of inactivation at various temperatures within the continuum registered in the matrix in the field. The modelling approach followed included (i) the recording of the temperature profile that the organism experiences in the field matrix, (ii) modelling the survival kinetics under isothermal conditions at a range of temperatures that were registered in the matrix in the field; and (iii) using the isothermal-based kinetic models to develop models for predicting survival under dynamic conditions. The time needed for 7 log CFU g^{-1} *Salmonella* Typhimurium in manure and manure-amended soil to reach the detection limit of the enumeration method (2 log CFU g^{-1}) under tropical conditions in the Central Agro-Ecological Zone of Uganda was predicted to be 61–68 days and corresponded with observed CFU of about 2·2–3·0 log CFU g^{-1}, respectively. The Bias and Accuracy factor of the prediction was 0·71–0·84 and 1·2–1·4, respectively.

Conclusions: Survival of *Salm*. Typhimurium under dynamic field conditions could be for 71–84% determined by the developed modelling approach, hence substantiating the working hypothesis.

Significance and Impact of the Study: Survival kinetic models obtained under isothermal conditions can be used to develop models for predicting the persistence of manure-borne enteric bacteria under dynamic field conditions in an agricultural environment.

Introduction

A number of factors including soil management type (Franz *et al.* 2005; Semenov *et al.* 2008), physical and chemical properties of the soil (Mubiru *et al.* 2000; Franz *et al.* 2008), cattle feeding regiment and manure composition (Franz *et al.* 2005), moisture condition and inoculum density (Ongeng *et al.* 2011a), background microbial

community (Kudva *et al.* 1998; Himathongkham *et al.* 1999; Jiang *et al.* 2002; You *et al.* 2006; van Overbeek *et al.* 2010; Semenov *et al.* 2010), the rhizosphere (Gagliardi and Karns 2002), aeration (Kudva *et al.* 1998), oxygen availability (Semenov *et al.* 2010) and temperature (Wang *et al.* 1996; Kudva *et al.* 1998; Himathongkham *et al.* 1999; Arrus *et al.* 2006; Semenov *et al.* 2007, 2010; Garcia *et al.* 2010) have been shown to affect the survival of manure-borne pathogenic bacteria such as *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in manure and in manure-amended soil. In reality, these factors do not act singly, but interact to determine the overall survival pattern. However, temperature is considered as the most explicit influential environmental factor due to the profound effect it has on the growth and decay rates of bacteria. Himathongkham *et al.* (1999) compared the survival of *E. coli* O157:H7 and *Salm*. Typhimurium in cow manure at 4, 20 and 37°C and demonstrated that the decay rate of the organisms increased with temperature. Wang *et al.* (1996) and Kudva *et al.* (1998) reported similar results studying survival of *E. coli* O157:H7 in bovine faeces at 5, 22 and 37°C and at 37, 45 and 70°C, respectively. In contrast, experiments performed by Jiang *et al.* (2002) on the survival of *E. coli* O157:H7 in manure-amended soil incubated at 5, 15 and 21°C demonstrated that the survival time of the organism increased with temperature.

None of the isothermal survival studies mentioned earlier went beyond describing temperature effects on survival. However, results of isothermal studies performed in the laboratory might become more meaningful when made relevant for practical applications in a real environment, e.g., prediction of survival under dynamic conditions in the field. One important question that has never been addressed is whether survival parameter(s) obtained under isothermal condition in the laboratory can be used to develop kinetic models for predicting persistence in the field. Semenov *et al.* (2007) compared the survival of *E. coli* O157:H7 and *Salm*. Typhimurium in manure between an isothermal set-up with four mean temperatures (7, 16, 23 and 33°C) and a nonisothermal set-up with two amplitudes of temperature oscillations (±4 and ±7°C) about the mean and observed that survival was significantly reduced in case of temperature oscillations compared to static conditions. These results suggest that the response of *E. coli* O157:H7 and *Salm*. Typhimurium to fluctuating temperatures cannot be deduced from their survival characteristics determined at one mean constant temperature only.

The objective of this study is to propose a new approach for using isothermal data to describe survival under dynamic conditions, e.g., in the field, i.e., (step i) determine the temperature profile that the organism experiences under dynamic conditions, (step ii) obtain and model the isothermal survival characteristics within the range of temperatures registered under dynamic conditions; and (step iii) use the kinetic models obtained under isothermal conditions to propose a model for predicting survival under dynamic conditions. Hereto, we hypothesize that the inactivation phenomena associated with survival profiles recorded for *E. coli* O157:H7-Rifr and *Salm*. Typhimurium-Rifr in manure and manure-amended soil under field and screen house conditions were as a result of the cumulative effect of inactivation at various temperatures within the continuum registered in the matrices. The previously indicated step (i) was performed and reported by Ongeng *et al.* (2011a): it was observed under tropical climatic conditions in the Central Agro-Ecological Zone (CAEZ) of Uganda that *E. coli* O157:H7 and *Salm*. Typhimurium experienced temperature fluctuations between 16 and 42°C during survival in manure and in manure-amended soil in the field and in the screen house. In a final step (step iv), the performance of the isothermally derived models to predict survival time under dynamic conditions is then evaluated using field and screen house survival data from Ongeng *et al.* (2011a).

Materials and methods

Isothermal survival experiments

A spontaneous rifampicin-resistant derivative of *Salmonella enterica* serovar Typhimurium LT2 (*Salm*. Typhimurium-Rifr) was used as test strain, while xylose lysine tergitol 4 agar medium (XLT4) incorporated with 100 μg ml^{-1} rifampicin, 50 μg ml^{-1} cycloheximide and 50 μg ml^{-1} nystatin (XLT4-Rif100-Cy50-Ny50) was used for CFU counting. XLT4-Rif100-Cy50-Ny50 was previously validated as a selective medium for enumeration of *Salm*. Typhimurium-Rifr in nonsterile manure-amended soil matrix (Ongeng *et al.* 2011b). The inoculum was prepared as described previously (Ongeng *et al.* 2011a). The manure used was fresh bovine manure obtained from Galloway beef cattle grazing on a meadow, while an Endohypostagnic Lixisol soil (FAO-ISRIC-ISSS 1998) obtained from the village of Nyabeda in Western Kenya was used as the soil matrix. Physicochemical properties of the soil were reported in a previous study (Pypers *et al.* 2006). Total aerobic CFU counts (mean ± SE, $n = 3$) in the soil and manure were 6·48 ± 0·75 and 9·34 ± 0·94 log CFU g^{-1}, respectively, as determined on LB agar following incubation of the plates at 30°C for 24–48 h. Contaminated manure was prepared by inoculating *Salm*. Typhimurium-Rifr at a rate of 7 log CFU g^{-1} and mixed thoroughly by kneading to distribute the inoculum.

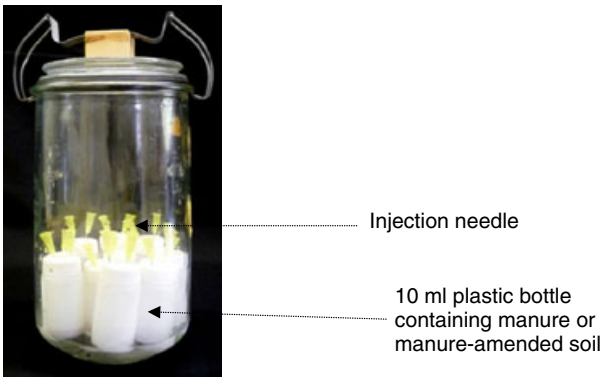

Figure 1 Illustration of the isothermal experimental set-up unit.

To prepare contaminated manure-amended soil, 100 g of manure was inoculated with *Salm*. Typhimurium-Rifr at a rate of 8 log CFU g^{-1} as described earlier followed by mixing inoculated manure with 900 g of soil to achieve a population size of 7 log CFU g^{-1} *Salm*. Typhimurium-Rifr in manure-amended soil matrix. Five grams of the inoculated matrices was dispensed in 10-ml plastic bottles fitted with injection needles to enable gas exchange. The bottles were placed in 2-l glass jars containing a small quantity of water that was sealed using rubber gaskets. Each jar contained 10 bottles (Fig. 1). The water in the glass jar was used to maintain the moisture constant at 80% in the matrices. The jars were then incubated at 16, 25, 37 and 42°C, respectively. Control matrices without inoculum were incubated under the same conditions. Each treatment was carried out in triplicate. The jars were opened weekly to avoid development of anoxic condition and to withdraw samples for microbiological analysis.

Microbiological analysis

At each sampling point, one bottle was removed from each replicate treatment. The content of each bottle was diluted in 45 ml of 0·9% saline and vortexed twice for 2 min. Tenfold dilution series were then prepared followed by spread plating in duplicate 100 μl of appropriate dilutions on XLT4-Rif100-Cy50-Ny50. CFU were enumerated following 24 h of incubating the plates at 37°C.

Primary modelling applied to the isothermal data

Cell counts (CFU g^{-1}) were log-transformed (log CFU g^{-1}) and fitted to the log-linear model (Bigelow and Esty 1920; eqn 1) using the Gina Fit Excel-Add-In model fitting tool (Geeraerd *et al.* 2005).

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

In eqn 1, N is CFU at any time (days), No is the initial CFU, k_{max} is the first-order inactivation rate constant (day^{-1}). The goodness-of-fit of survivor curves were assessed using the root mean sum of squared error (RMSE), ranging between 0·30 and 0·61, and the adjusted coefficient of determination (Adj-R^2), ranging between 0·80 and 0·96. While a Weibull model (two parameters) was also tested using Gina Fit and showed slightly better goodness-of-fit criteria for five of the eight survival curves (RMSE ranging between 0·30 and 0·55 and Adj-R^2 between 0·85 and 0·96), it was decided to proceed with the log-linear model as it is the most simple one, necessitating only one parameter and hence only one secondary model. The adequacy of the model fit was evaluated further by looking at the confidence interval and predicted interval of the fitted data points. The *lsqnonlin* procedure of the MatLab Optimization Toolbox (The Mathworks Inc., ver. 2007b; www.mathworks.com) was used for this purpose.

Secondary modelling and prediction of bacterial survival under dynamic conditions in the field and in the screen house

Secondary models were developed to describe temperature dependency of the decline rate of *Salm*. Typhimurium-Rifr in manure and manure-amended soil. First, values of k_{max} were transformed to natural logarithm and fitted to the Arrhenius model (eqn 2):

$$\ln k_{max} = \ln k_o - \frac{E_A}{RT} \quad (2)$$

where k_{max} is the decline rate at any temperature (day^{-1}), k_o is the inactivation rate at reference temperature (day^{-1}), E_A is the activation energy (kJ mol^{-1}), R is the molar gas constant ($8{\cdot}314 \times 10^{-3}$ kJ mol^{-1} K^{-1}) and T is the absolute temperature (K). As will be shown in the results section, Arrhenius transformation did not provide any functional relationship within the temperature range investigated. Values of k_{max} were then plotted directly against temperature without transformation to determine temperature dependency of the decline rate and to derive a secondary model. The decline rates under dynamic conditions in the field or in the screen house were generated by filling out the instantaneous values of T that the organism experienced in the matrices in the secondary model. The instantaneous values of T were derived from the hourly measurements of temperature recorded during the survival of *Salm*. Typhimurium-Rifr in manure and manure-amended soil under field conditions or in the

screen house in the CAEZ of Uganda (Ongeng *et al.* 2011a). Hours were converted to days before use. The decline rates calculated as earlier were then used to reconstruct CFU counts as a function of time under field conditions or in the screen house according to eqn 3, from where it is assumed that the log-linear model from isothermal experiments (Section 2·3) is transferable to the dynamic conditions.

$$\log N_i = \log N_p - \left[\frac{k_{max}.t_d}{\ln(10)}\right] \quad (3)$$

In eqn 3, N_i is the population at any time instant (CFU g^{-1}), N_p is the population at a previous time instant (CFU g^{-1}), k_{max} is the decline rate (day^{-1}) at temperature T corresponding with the time instant N_p is present and t_d is the elapsed time between two temperature measurements (which is always equal to 1 h or 0·042 days). The reconstructed CFU counts are a model prediction of the survival of *Salm*. Typhimurium-Rifr in manure and manure-amended soil under dynamic conditions in the field and in the screen house. Model predictions of the time for CFU number of *Salm*. Typhimurium-Rifr in manure and manure-amended soil to reach the detection limit (ttd) of the plate count method (2 log CFU g^{-1}) were then obtained. Predictions were performed only for survival in matrices maintained at high moisture level in the field and in the screen house, and in matrices exposed to exclusive field conditions based on results of previous survival experiments reported by Ongeng *et al.* (2011a).

Performance evaluation of the predictive model

The Bias factor (B_f) and the Accuracy factor (A_f) indices developed by Ross (1996) for assessing the performance of predictive models in food microbiology were used as a starting point to evaluate the performance of the isothermally derived model to predict survival under dynamic conditions. In this study, the B_f and A_f is defined by eqns 4 and 5, respectively:

$$B_f = 10^{\left[\sum \log\left(\frac{ttd_p}{ttd_o}\right)/n\right]} \quad (4)$$

$$A_f = 10^{\left[\sum \left|\log\left(\frac{ttd_p}{ttd_o}\right)\right|/n\right]} \quad (5)$$

where B_f is the bias factor, A_f is the accuracy factor, ttd_p is the predicted time (days) for CFU number to reach the detection limit of the plate count method (2 log CFU g^{-1}), ttd_o is the observed time for CFU to reach the detection limit of the plate count method and n is the number of observations used in the calculation. As applied in this study, B_f is a measure of over- or underprediction of the ttd, while A_f is a measure of the spread of the observed ttd about the predicted value. By eqn 4, a B_f of 1 indicates that isothermally derived model exhibits no systematic over- or under prediction of ttd under dynamic conditions; a $B_f < 1$ signifies that isothermally derived model underestimates survival under dynamic conditions ('fail dangerous') and a $B_f > 1$ means that the isothermally derived model overestimates survival under dynamic conditions ('fail safe'). With eqn 5, an A_f of 1 indicates perfect agreement between all predicted and observed ttd values, and an $A_f > 1$ (e.g., 1·4) shows that the prediction is on average, either 40% smaller or 40% larger than the observed values. The predicted ttd values were compared with the ttd values determined experimentally in the field and in the screen house as described by Ongeng *et al.* (2011a). The original Bias and Accuracy factors were derived for assessing the growth rate or generation time predictions. In this study, these performance indices were applied on ttd prediction because of the need to precisely and accurately predict the persistence of *E. coli* O157:H7 and *Salm*. Typhimurium to determine the length of time manure can be kept before incorporation into the soil or when vegetables can be cultivated when raw manure is amended to soil to ensure the safety of the vegetables preharvest.

Results

Survival of *Salmonella* Typhimurium-Rifr under isothermal conditions and primary modelling

The isothermal survival patterns of *Salm*. Typhimurium-Rifr in manure and manure-amended soil recorded at different set temperatures are shown in Fig. 2. At all temperatures investigated, CFU number of *Salm*. Typhimurium-Rifr declined with time except in manure samples held at 16°C where cell counts of the organism stabilized for 1 week before decline in CFU counts became observable. The decline was apparently temperature dependent. In general, the order of decline with respect to temperature was $42 > 25 \geq 37 > 16$°C. The log-linear model adequately fitted the survivor curves. RMSE values ranged from 0·30 to 0·61, while Adj-R^2 values were in the range of 0·80–0·96. The 95% confidence and predicted intervals of CFU counts during survival according to the log-linear model are illustrated in Figs 3 and 4 for manure and manure-amended soil, respectively.

Secondary modelling and prediction of bacterial survival in the field and in the screen house

Attempts to derive temperature dependency of k_{max} within the temperature range experimented according to

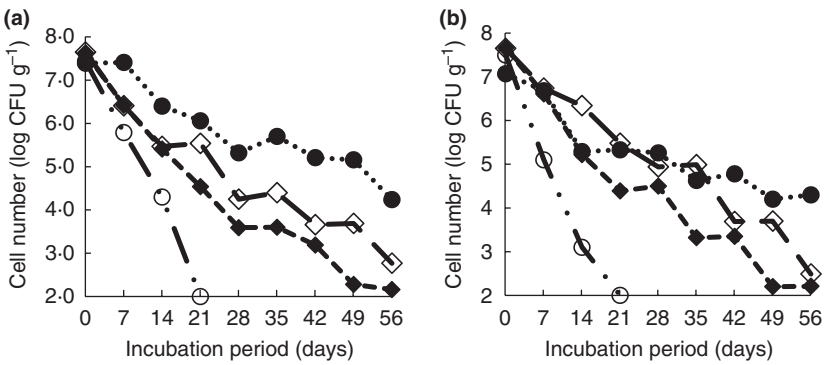

Figure 2 Survival of *Salmonella* Typhimurium-Rifr in manure (a) and manure-amended soil (b) at various temperatures. (●): 16°C; (◆): 25°C; (◇):37°C; (○): 42°C. Data points are averages of three replicates. Error bars are not shown for clarity of illustration.

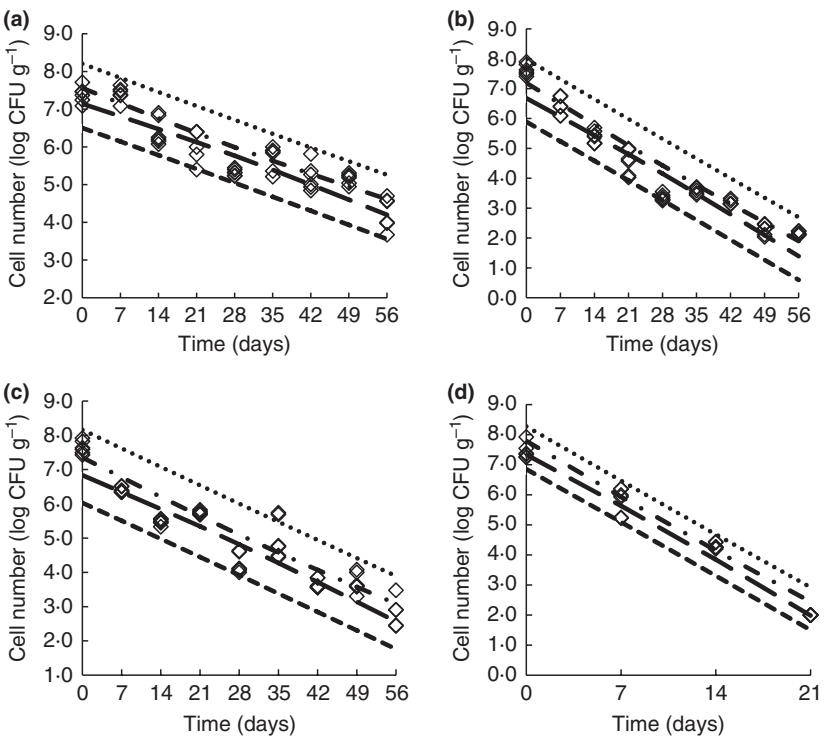

Figure 3 Confidence interval (two inner lines) and prediction interval (two outer lines) of the fitted survival curve of *Salmonella* Typhimurium-Rifr inoculated at 7 log CFU g^{-1} in manure at various temperatures according to the log-linear model. (◇): Cell number (log CFU g^{-1}); (a): 16°C; (b): 25°C; (c): 37°C; (d): 42°C. All replicates are shown.

Arrhenius method (eqn 2) did not provide any functional linear relationship. Arrhenius plot for the data is shown in Fig. 5. Graphical illustration of the decline rate as a function of temperature is presented in Fig. 6. In general, for both manure and manure-amended soil, the decline rate increased by a factor of 2 from 16 to 25°C, remained fairly constant between 25 and 37°C, and then increased by a factor of 3 from 37 to 42°C. As the decline rate apparently did not change between 25 and 37°C, the decline rate within that continuum was approximated to be equal to the average of the decline rates obtained at 25 and 37°C (separately for manure and manure-amended soil). This suggested a set of three sequential linear equations with similar but not identical parameter values for both manure and manure-amended soil, i.e., (i) a linear increase in decline rate between 16 and 25°C (eqns 6 and 9), (ii) a more or less constant decline rate between 25 and 37°C (eqns 7 and 10) and (iii) a drastic linear increase in decline rate between 37 and 42°C (eqns 8 and 11). The sequential linear equations are corresponding to a piecewise linear interpolation procedure using four data points, namely (i) the observed decline rate at 16°C, (ii) the average of the decline rates at 25 and 37°C, at 25°C, (iii) the average of the decline rates at 25 and 37°C, at 37°C and (iv) the observed decline rate at 42°C. This means that six parameters (three subintervals × two parameters per subinterval) need to be identified. By imposing that the linear equations should match the data

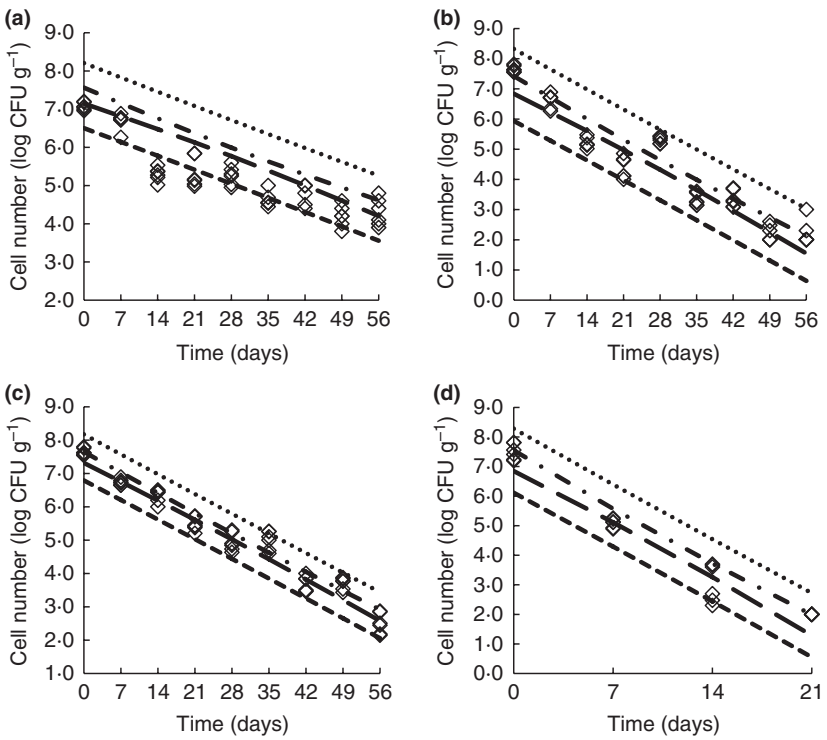

Figure 4 Confidence interval (two inner lines) and prediction interval (two outer lines) of the fitted survival curve of *Salmonella* Typhimurium-Rifr inoculated at 7 log CFU g^{-1} in manure-amended soil at various temperatures according to the log-linear model. (◇): Cell number (log CFU g^{-1}); (a): 16°C; (b): 25°C; (c): 37°C; (d): 42°C. All replicates are shown.

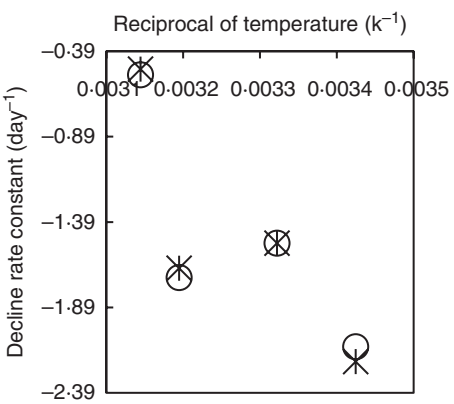

Figure 5 The effect of temperature on decline rate of *Salmonella* Typhimurium-Rifr according to Arrhenius model. (✳): manure; (○): manure-amended soil.

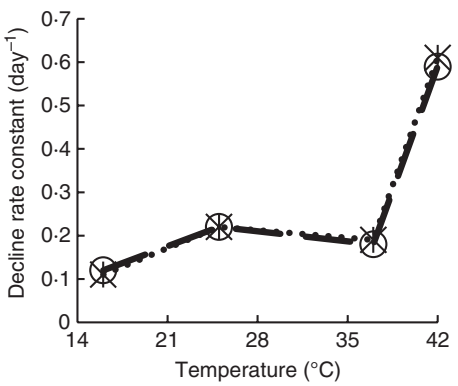

Figure 6 Decline rate of *Salmonella* Typhimurium-Rifr survival in manure and manure-amended soil at isothermal temperatures. (✳): manure; (○): manure-amended soil.

points at either end of its subinterval, the six parameters are fixed. Such linear interpolation procedure is common practice in *table lookup strategies* where a measurement with a very high frequency is recorded and is being interpolated for use to model another, usually much less frequently measured, variable (as in, for example, Bernaerts *et al.* 2002). It is much less common as a secondary model but was chosen here for two reasons: (i) it is a simple model correctly describing the observations; and (ii) a second- or third-order polynomial for these four data points would lead to unrealistic curvatures. Graphical illustration of the decline rate as determined by experiment and fitted according to the secondary model (eqns 6–8: manure; eqns 9–11: manure-amended soil) is presented in Fig. 7

$$k_{\max} = 0 \cdot 008376T - 0 \cdot 012883 \quad [16 \geq T \leq 25^{\circ}\mathrm{C};\ \text{manure}] \tag{6}$$

$$k_{\max} = 0 \cdot 19561 \quad [25 \geq T \leq 37^{\circ}\mathrm{C};\ \text{manure}] \tag{7}$$

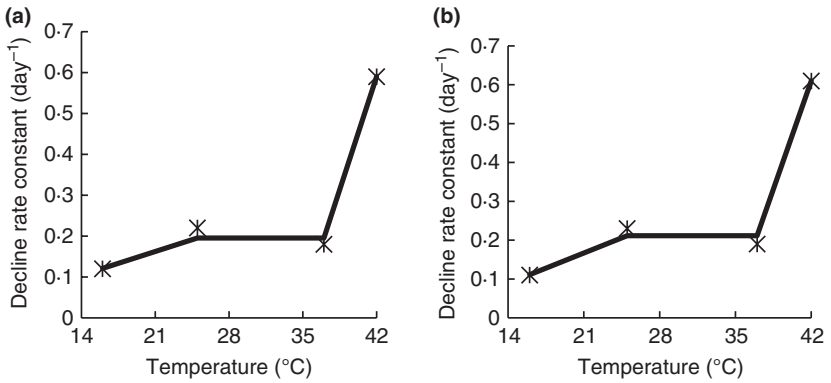

Figure 7 Decline rate of *Salmonella* Typhimurium in manure and manure-amended soil as experimentally determined and according to the secondary models. (a): manure; (b) manure-amended soil; (✳): experimentally determined; (—): secondary model.

$$k_{max} = 0{\cdot}064065T - 2{\cdot}173881 \quad [37 \geq T \leq 42^{\circ}C;\ \text{manure}] \tag{8}$$

$$k_{max} = 0{\cdot}0111115T - 0{\cdot}066609 \quad [16 \geq T \leq 25^{\circ}C;\ \text{manure-amended soil}] \tag{9}$$

$$k_{max} = 0{\cdot}21127 \quad [25 \geq T \leq 37^{\circ}C;\ \text{manure-amended soil}] \tag{10}$$

$$k_{max} = 0{\cdot}103510T - 3{\cdot}618599 \quad [37 \geq T \leq 42^{\circ}C;\ \text{manure-amended soil}] \tag{11}$$

In eqns 6–11, k_{max} is the decline rate (day^{-1}) and T is the temperature (°C). Model predictions for the survival of *Salm*. Typhimurium-Rifr in manure and manure-amended soil under dynamic conditions in the field and in the screen house based on reconstruction according to eqns 3 and 6–11, making use of the measured temperature profiles, are shown in Fig. 8. The time needed for CFU number of *Salm*. Typhimurium-Rifr to reach the detection limit of the plate count method (2 log CFU g^{-1}) in manure and manure-amended soil in the field is predicted to be 63–68 and 62 days, and 65 and 61 days in the screen house, respectively.

Performance evaluation of the predictive model

Graphical illustration of the survivor curves as described by isothermally derived model and the data points determined experimentally (Ongeng *et al.* 2011a) are shown in Fig. 9. The B_f and A_f of the isothermally derived prediction model describing the survival of *Salm*. Typhimurium-Rifr in manure under field and screen house conditions was 0·84 and 1·2, respectively. At the predicted ttd points, the observed CFU numbers of *Salm*. Typhimurium-Rifr in manure were around 2·2 log CFU g^{-1} both under screen house and field conditions (Fig. 9a,b,e). The isothermally derived model underestimated ttd for *Salm*. Typhimurium-Rifr in manure by 12 and 14 days for matrix maintained at high moisture level and matrix exposed to exclusive field conditions, respectively, and by 12 days for manure maintained at high moisture level in the screen house. The B_f and A_f of the isothermally derived model used to predict the survival of *Salm*. Typhimurium-Rifr in manure-amended soil under field and screen house conditions was 0·71 and 1·4, respectively. These performance index values were associated with about 3 log CFU g^{-1} of *Salm*. Typhimurium-Rifr still present in the matrix (Fig. 9c,d,f). The isothermally derived model underestimated ttd for *Salm*. Typhimurium-Rifr in manure-amended soil by 22 days in the field irrespective of the moisture condition and by 30 days for manure-amended soil maintained at high moisture level in the screen house.

Discussion

In this study, we showed for the first time that kinetic models obtained under isothermal conditions can be used to develop models for predicting the persistence of enteric bacteria under dynamic conditions, e.g., in the field and in the screen house. We particularly assessed the extent to which the decline rates of *Salm*. Typhimurium-Rifr in manure and manure-amended soil obtained under isothermal conditions could be used to predict survival under field and screen house conditions in the CAEZ of Uganda. The log-linear model adequately described survival data. It was interesting to note that the decline rate was only limitedly affected by matrix type. This is in contrast with the findings of Jiang *et al.* (2002) which showed that enteric bacteria declined faster in manure than in manure-amended soil under isothermal conditions. Differences in strain and physicochemical properties of the manure and soil might explain the discrepancies between our results and those of Jiang *et al.* (2002).

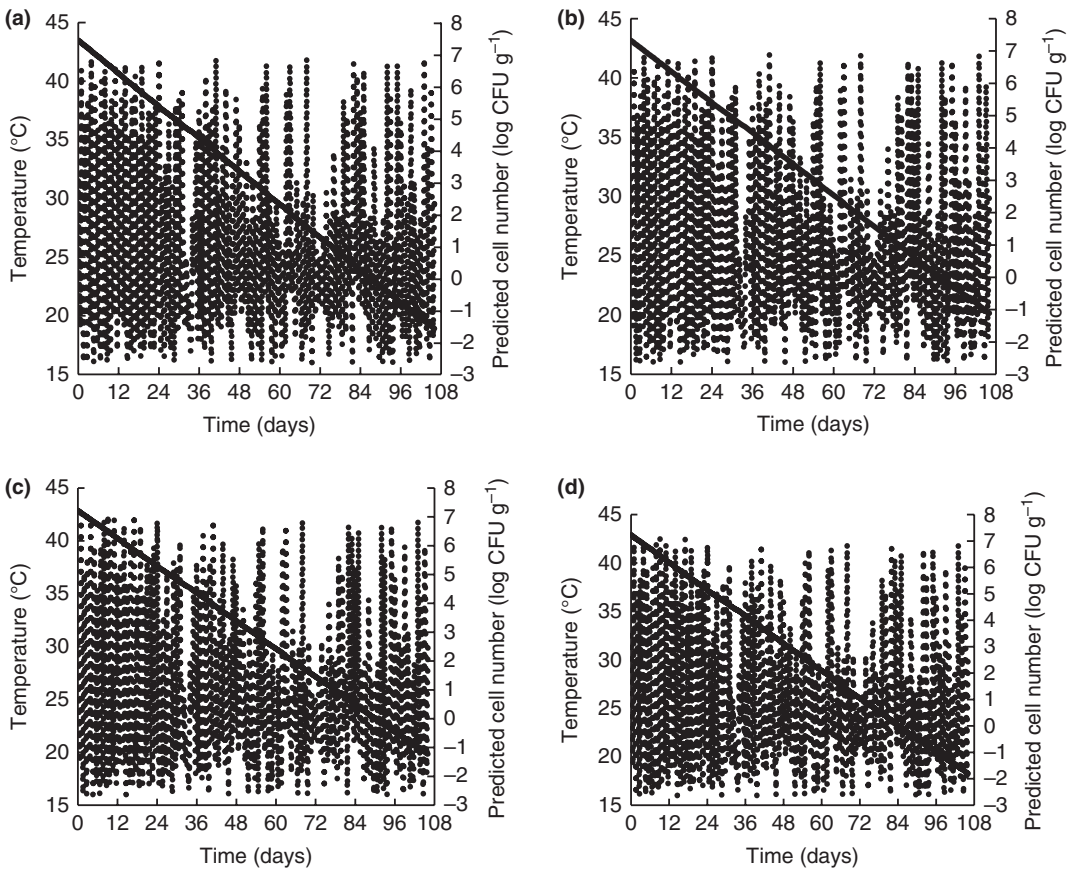

Figure 8 Isothermally derived decline curve describing the survival of *Salmonella* Typhimurium-Rifr in manure and manure-amended soil under dynamic conditions in the field or screen house. (—): isothermally derived decline curve; (•••): temperature; (a): manure in the field; (b): manure-amended soil in the field; (c): manure in the screen house; (d) manure-amended soil in the screen house.

Attempts to derive temperature dependency of the decline rate within the temperature limits used in this study according to Arrhenius transformation did not provide any functional relationships, and therefore, no secondary model could be derived based on the Arrhenius model. Failure of Arrhenius relationship as observed in this study has also been reported for temperature effects in food systems (Robinson *et al.* 1998; Ongeng *et al.* 2007). The Arrhenius model has been derived empirically based on a thermodynamic assumption that the activation energy is independent of temperature which may not be valid for biological systems (McMeekin *et al.* 1992). On the other hand, lack of a linear relationship between the decline rate and temperature as observed in this study is inconsistent with the results of a previous study (Himathongkham *et al.* 1999). One peculiar observation from our study was the manner in which the decline rate varied with temperature. First, the decline rate increased with temperature within the range of 16–25°C. This could be accounted on the basis that at 16°C, inactivation was delayed as the cells were less active and therefore less stressed by the harsh environment, but decline rate increased at 25°C because cells are expected to be more active and therefore biotic stresses (e.g., inter- and intra-species competition for substrates, production of antimicrobial substances by antagonistic flora) become more pronounced. Secondly, the decline rate apparently remained stable around 0·2 day^{-1} between 25 and 37°C. This could probably mean that there was a balance between growth and inactivation, but growth was probably more pronounced around 37°C because it is the optimal temperature for growth of *Salmonella* spp. Thirdly, the decline rate increased rapidly from 37 to 42°C probably because inactivation outweighed growth: 42°C is already above the optimal physiological temperature for growth of the organism. These phenomena constituted the basis for the formulation of the three-stage secondary models that were subsequently used to derive decline rates for the field and screen house environmental conditions.

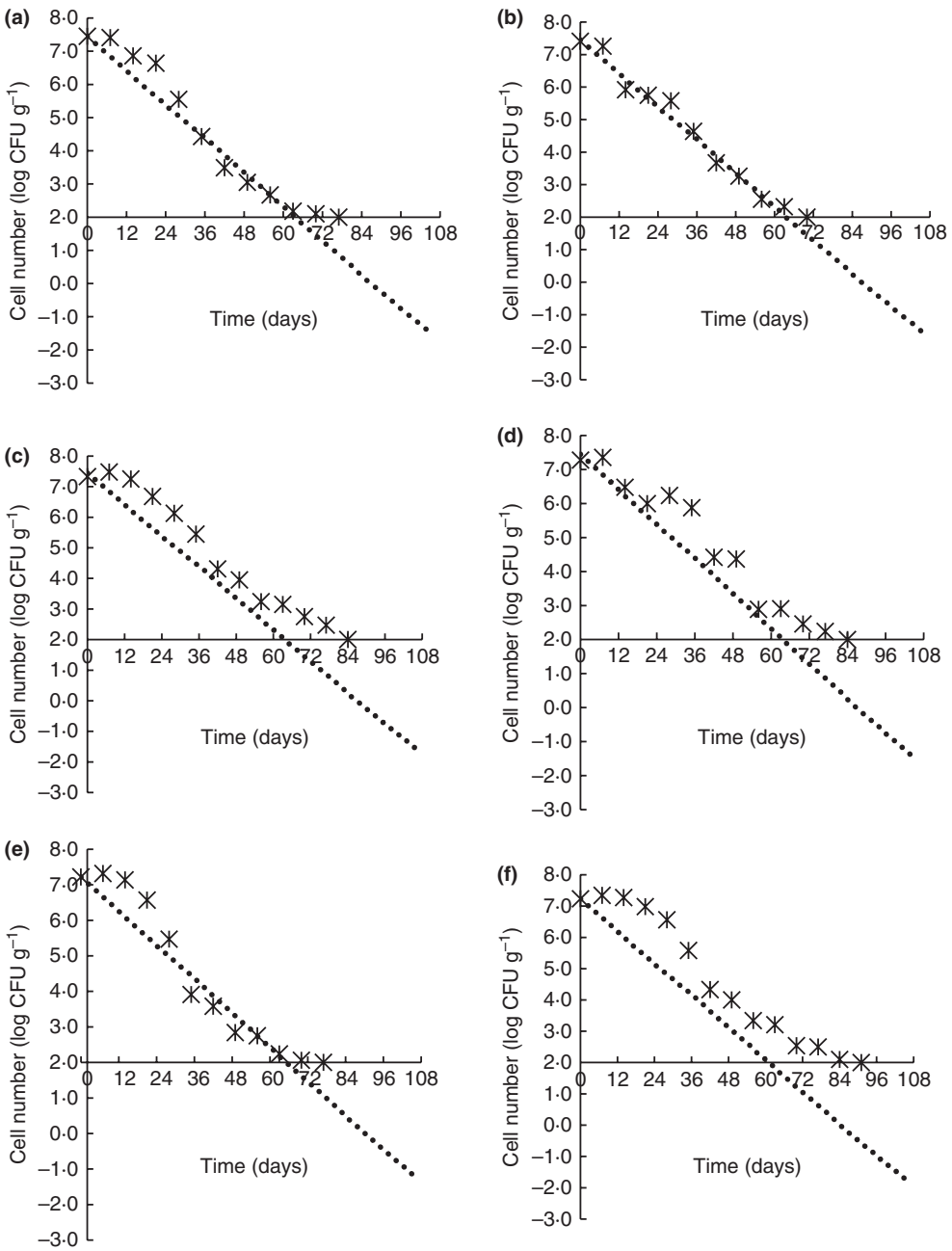

Figure 9 Survivor curves of *Salmonella* Typhimurium-Rifr in manure and manure-amended soil under field and screen house conditions as predicted by isothermally derived model (•••) and experimentally determined (✳; Ongeng *et al.* 2011a). (a): manure maintained at high moisture level in the field; (b): manure exclusively exposed to field conditions; (c): manure-amended soil maintained at high moisture level in the field; (d): manure-amended soil exclusively exposed to field conditions; (e): manure maintained at high moisture level in the screen house; (f): manure-amended soil maintained at high moisture level in the screen house. Experimental data are averages of three replicates.

The isothermal experiment was performed using high moisture matrices. In principle, therefore, the isothermally derived prediction models were only suitable for predicting survival of *Salm*. Typhimurium-Rifr in matrices maintained at high moisture level in the field and in the screen house. However, the models were also deemed suitable for predicting survival in matrices exposed to exclusive field conditions. This was based on the results of the previous field experiment which showed that *Salm*. Typhimurium-Rifr inoculated at a rate of 7 log

CFU g^{-1} in manure or manure-amended soil took similar time periods in matrices that were maintained at high moisture level and in matrices that were exposed to exclusive field conditions to reach the detection limit of the direct plate count method (Ongeng *et al.* 2011a). Performance evaluation using the B_f index revealed that the isothermally derived prediction models underestimated the time *Salm*. Typhimurium-Rifr took in manure and manure-amended soil to reach the detection limit of the plating method. This is clearly illustrated in Fig. 9. The A_f index of 1·2 and 1·4 indicates that the predicted survival time was on average 20 and 40% shorter than the observed survival time for manure and manure-amended soil, respectively. Still, we believe that a model prediction for over/more than 60 days under realistic conditions, using only the following pieces of information: (i) level of starting population, (ii) survival kinetics obtained under isothermal conditions and (iii) the registered real temperature data, and yielding a prediction of 2 logs in comparison with an observed 2·2 logs (manure) or 3 logs (manure–soil), can be considered as being very accurate (manure) to rather accurate (manure-amended soil) – the error in any viable count method is *c.* ±0·3 log CFU (Jarvis 1989). The B_f of 0·84 and 0·71 suggests that the survival time till 2 logs of *Salm*. Typhimurium-Rifr in manure and manure-amended soil under field and screen house conditions was in general 84 and 71%, respectively, determined by temperature that the organism experienced in the matrices during *c.* 60 days. The remaining 16 and 29% of the survival time unaccounted for by the model could be due to other factors not included in the model. Therefore, inclusion of other factors that affect survival in the model might improve performance. For example, Mejlholm *et al.* (2010) showed that a complex model incorporating nine environmental parameters performed better than a less complex model in predicting the growth of *Listeria monocytogenes* in ready-to-eat meat and sea food. On the other hand, differences in physicochemical and biotic characteristics between manure and soil used in the isothermal experiment and those used in the field study might also be responsible for the discrepancies between the observed and predicted survival times for manure–soil conditions. In another study, Semenov *et al.* (2007) used survival data obtained from laboratory experiments conducted with a set of fixed oscillating temperatures (four mean temperatures:7, 16, 23 and 33°C with 2 amplitudes, ±4 and ±7°C, and with zero amplitude as control) to construct secondary models that relate rate of change of CFU of *E. coli* O157:H7 or *Salm*. Typhimurium in cattle manure with mean temperature and temperature amplitude. The authors suggested that the secondary models could be used as a risk assessment tool to predict survival of the pathogens in natural ecosystems such as farmyard manure in which temperature is always dynamic. Considering that the models of Semenov *et al.* (2007) do not relate CFU with temperature, but instead relate the rate of change of CFU with mean temperature and temperature amplitude, those models may not reliably be used to predict survival in natural ecosystems as suggested by the authors because temperature regimes in nature are unlikely to be fixed at the oscillatory ranges used in the laboratory experiment. In contrast, however, the models developed in this study provide a feasible option to predict survival based on the relationship between CFU and temperature that the organism experiences in an ecosystem.

Predictive modelling is less developed within the realm of environmental microbiology. As such, there are no official criteria for determining the acceptability of a model based on performance evaluation results. In predictive food microbiology, various nonagreed criteria have been used to designate acceptability of models. Ross (1999) recommended that B_f in the range of 0·9–1·05 be considered good, 0·7–0·9 or 1·06–1·15 considered acceptable and <0·7 or >1·15 considered unacceptable. Armas *et al.* (1996) on the other hand considered B_f in the range of 0·6–3·99 as being acceptable. Thurette *et al.* (1998) considered predictions as being satisfactory if the observed and predicted growth differed by <0·5 log CFU g^{-1}. In absence of official criteria, Mellefront *et al.* (2003) suggested that results of performance evaluation should be interpreted in a manner consistent with the situation in which the predictive model is likely to be applied. Although the B_f indices indicate that the isothermally derived models are 'fail dangerous', from food safety perspective, it is unlikely that the residual cell population slightly underestimated by the isothermally derived predictive models (respectively, at 2·2 and 3 logs) developed in this study may pose microbiological safety problems at harvest if vegetables are cultivated on soil amended with manure containing 7 log CFU g^{-1} *Salm*. Typhimurium 61–68 days postmanure amendment. This argument is substantiated by results of plant contamination studies (Ongeng *et al.* 2011c) which show that cultivation of cabbage on manure-amended soil inoculated with 4 log CFU g^{-1} *Salm*. Typhimurium-Rifr did not result in vegetable contamination at harvest (120 days post-transplantation). The 7 log CFU g^{-1} inoculum density used to develop the prediction models may be unrealistically high (Franz *et al.* 2007). On the other hand, if lower inoculum concentration such as 4 log CFU g^{-1} presents a more realistic contamination level expected in practice, then using the same prediction models, it is clear that the time to achieve 4 log reduction in the cell counts of the 7 log CFU g^{-1} inoculum (40–42 days; Fig. 9) would be equivalent to complete reduction

in the cell counts of the 4 log CFU g^{-1} inoculum as determined in a previous field experiment (Ongeng *et al.* 2011a). Hence, the predicted survival time of 61–68 days for the 7 log CFU g^{-1} inoculum would provide a conservative 'fail safe' estimate for the time that should elapse between the application of untreated manure containing 4 log CFU g^{-1} inoculum and cultivation of vegetables.

Conclusions

This study showed for the first time that results of survival experiments performed under isothermal conditions in the laboratory can be used for practical applications under dynamic conditions in the field. This was demonstrated with *Salm*. Typhimurium-Rifr through development of isothermally derived predictive models by combining data on decline rate parameter obtained at various isothermal temperatures and data on temperature profile that the organism experienced in manure and manure-amended soil under field conditions in the CAEZ of Uganda. Performance evaluation of the isothermally derived prediction models showed that the models predicted very accurately (for manure) to rather accurately (for manure-amended soil) the population level remaining after more than 60 days when starting from high contamination levels. From food safety perspective, the models are deployable to predict the survival time of *Salm*. Typhimurium-Rifr in manure and manure-amended soil under tropical conditions in the CAEZ of Uganda for realistic, low contamination levels. A major limitation of this study was that only one soil type and a single bacterial strain were used. Future studies should validate the models with different strains, various soil types and data obtained under various field conditions.

Acknowledgements

This research was supported by International Foundation for Science (Ref. C/4294-1) and Belgian Development Agency (UNI2006/01).

References

Armas, A.D., Wynne, A. and Sutherland, J.P. (1996) Validation of predictive models using independently published data. Abstract. 2nd International Conference of Predictive Microbiology, Hobart, Tas., Australia.

Arrus, K.M., Holley, R.A., Ominski, K.H., Tenuta, M. and Blank, G. (2006) Influence of temperature on *Salmonella* survival in hog manure slurry and seasonal temperature profiles in farm manure storage reservoirs. *Livest Sci* **102**, 226–236.

Bernaerts, K., Servaes, R.D., Kooyman, S., Versyck, K.J. and Van Impe, J.F. (2002) Optimal temperature input design for estimation of the square root model parameters: parameter accuracy and model validity restrictions. *Int J Food Microbiol* **73**, 145–157.

Bigelow, W.D. and Esty, J.R. (1920) The thermal death point in relation to typical thermophylic organisms. *J Infect Dis* **27**, 602.

FAO-ISRIC-ISSS (1998). *World Reference Base for Soil Resources*. World soil Resources Report nr. 84, pp. 88. Rome: FAO.

Franz, E., van Diepeningen, A.D., de Vos, O.J. and van Bruggen, A.H.C. (2005) Effects of cattle feeding regimen and soil management type on the fate of *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium in manure, manure-amended soil, and lettuce. *Appl Environ Microbiol* **71**, 6165–6174.

Franz, E., Visser, A.A., van Diepeningen, A.D., Klerks, M.M., Termoshuizen, A.J. and van Bruggen, A.H.C. (2007) Quantification of contamination of lettuce by GFP-expressing *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium. *Food Microbiol* **24**, 106–112.

Franz, E., Semenov, A.V., Termorshuizen, A.J., de Vos, O.J., Bokhorst, J.G. and van Bruggen, A.H. (2008) Manure-amended soil characteristics affecting the survival of *E. coli* O157:H7 in 36 Dutch soils. *Environ Microbiol* **10**, 313–327.

Gagliardi, J.V. and Karns, J.S. (2002) Persistence of *Escherichia coli* O157:H7 in soil and on plant roots. *Environ Microbiol* **4**, 89–96.

Garcia, R., Baelum, J., Fredslund, L., Santorum, P. and Jacobsen, C.S. (2010) Influence of temperature and predation on survival of Salmonella enterica serovar Typhimurium and expression of invA in soil and manure-amended soil. *Appl Environ Microbiol* **76**, 5025–5031.

Geeraerd, A.H., Valdramidis, V.P. and Van Impe, J.F. (2005) GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *Int J Food Microbiol* **102**, 95–105.

Himathongkham, S., Bahari, S., Riemann, H. and Cliver, D. (1999) Survival of *Escherichia coli* O157:H7 and *Salmonella* Typhimuriun in cow manure and cow manure slurry. *FEMS Microbiol Lett* **178**, 251–257.

Jarvis, B. (1989) *Statistical Aspects of the Microbiological Analysis of Foods*. Amsterdam: Elsevier.

Jiang, X., Morgan, J. and Doyle, M.P. (2002) Fate of *Escherichia coli* O157:H7 in manure-amended soil. *Appl Environ Microbiol* **68**, 2605–2609.

Kudva, I.T., Blanch, K. and Hovde, C.J. (1998) Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Appl Environ Microbiol* **64**, 3166–3174.

McMeekin, T.A., Ross, T. and Olley, J. (1992) Application of predictive microbiology to assure the quality and safety of fish and fish products. *Int J Food Microbiol* **15**, 13–32.

Mejlholm, O., Gunvig, A., Borggaard, C., Blom-Hanssen, J., Mellefront, L., Ross, T., Leroi, F., Else, T. *et al.* (2010) Predicting growth rates and growth boundary of *Listeria monocytogenes*: an international validation study with focus

on processed and ready-to-eat meat and seafood. *Int J Food Microbiol* **141**, 137–150.

Mellefront, L.A., McMeekin, T.A. and Ross, T. (2003) Performance evaluation of a model describing the effects of temperature, water activity, pH and lactic acid concentration on the growth of *Escherichia coli*. *Int J Food Microbiol* **82**, 45–58.

Mubiru, D.N., Coyne, M.S. and Grove, J.H. (2000) Mortality of *Escherichia coli* O157:H7 in two soils with different physical and chemical properties. *J Environ Qual* **29**, 1821–1825.

Ongeng, D., Ryckeboer, J., Vermeulen, A. and Devlieghere, F. (2007) The effect of micro-architectural structure of cabbage substratum and or background bacterial flora on the growth of *Listeria monocytogenes*. *Int J Food Microbiol* **119**, 291–299.

Ongeng, D., Muyanja, C., Ryckeboer, J., Geeraerd, A. and Springael, D. (2011a) Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in manure and manure-amended soil under tropical climatic conditions in Sub-Saharan Africa. *J Appl Microbiol* **110**, 1007–1022.

Ongeng, D., Muyanja, C., Ryckeboer, J., Geeraerd, A.H. and Springael, D. (2011b) Development and validation of a culture-based method suitable for monitoring environmental survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in developing countries. *Ann Microbiol* doi: 10.1007/s13213-011-0199-4.

Ongeng, D., Muyanja, C., Ryckeboer, J., Geeraerd, A. and Springael, D. (2011c) Transfer and internalisation of *E. coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in cabbage cultivated on contaminated manure-amended soil under tropical field conditions in Sub-Saharan Africa. *Int J Food Microbiol* doi: 10.1016/j.ijfoodmicro.2011.01.018.

van Overbeek, L.S., Franz, E., Semenov, A.V., de Vos, O.J. and van Bruggen, A.H. (2010) The effect of the native bacterial community structure on the predictability of *E. coli* O157:H7 survival in manure-amended soil. *Lett Appl Microbiol* **50**, 425–430.

Pypers, P., Delrue, J., Diels, J., Smolders, E. and Merckx, R. (2006) Phosphorus intensity determines short-term P uptake by pigeon pea (*Cajanus cajan L.*) grown in soils with differing P buffering capacity. *Plant Soil* **284**, 217–227.

Robinson, T.P., Ocio, M.J., Kaloti, A. and Mackey, B.M. (1998) The effect of growth environment on the lag phase of *Listeria monocytogenes*. *Int J Food Microbiol* **44**, 83–92.

Ross, T. (1996) Indices for performance evaluation of predictive models in food microbiology. *J Appl Bacteriol* **81**, 501–508.

Ross, T. (1999) *Predictive Food Microbiology Models in the Meat Industry*. Sydney, Australia: Meat and Livestock Australia. pp. 196.

Semenov, A.V., van Bruggen, A.H.C., van Overbeek, L., Termorshuizen, A.J. and Semenov, A.M. (2007) Influence of temperature fluctuations on *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in cow manure. *FEMS Microbiol Ecol* **60**, 419–428.

Semenov, A.V., Franz, E., van Overbeek, L., Termorshuizen, A.J. and van Bruggen, A.H. (2008) Estimating the stability of *Escherichia coli* O157:H7 survival in manure-amended soils with different management histories. *Environ Microbiol* **10**, 1450–1459.

Semenov, A.V., Franz, E. and van Bruggen, A.H.C. (2010) COLIWAVE: a simulation model for survival of *E. coli* O157:H7 in dairy manure and manure-amended soil. *Ecol Modell* **221**, 599–609.

Thurette, J., Membre, J.M., Ching, L.H., Tailliez, R. and Catteau, M. (1998) Behaviour of *Listeria* spp. in smoked fish products affected by liquid smoke, NaCl concentration, and temperature. *J Food Prot* **61**, 1475–1479.

Wang, G., Zhao, T. and Doyle, M.P. (1996) Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine faeces. *Appl Environ Microbiol* **62**, 2567–2570.

You, Y., Rankin, S.C., Aceto, H.W., Benson, C.E., Toth, J.D. and Dou, Z. (2006) Survival of *Salmonella enterica* serovar Newport in manure and manure-amended soils. *Appl Environ Microbiol* **72**, 5777–5783.