APPENDIX: The model

This appendix provides supplementary information on the model. It is not necessary to read this section to use Perfringens Predictor.

A.1 The evolution of Perfringens Predictor

Since the early 1980s, with the advent of powerful PCs, food safety research has increasingly turned to quantitative, computational tools. The development of databases, hand in hand with mathematical and statistical processing, has become a key element in food safety. This principle was recognised by the UK Food Standards Agency (formerly the Ministry of Agriculture Fisheries and Food), who launched their Predictive Microbiology Programme at the end of the 1980s. Work funded under this Programme produced a large amount of growth and survival data for various food-borne organisms and formed the underlying database for the *Food MicroModel* (FMM) software. These data now constitute the core of the database, *ComBase* (www.combase.cc) which underlies the *Combase Predictor* (www.combase.cc/predictor.html) predictive microbiology tool which supersedes FMM and predicts the growth and survival of microorganisms in food.

Perfringens Predictor, is a further development in this series of computer programmes for microbiological safety. It is also funded by the UK Food Standards Agency, is free of charge to the end user and is based on many of the same principles as *Combase Predictor* but has been specifically developed and validated to predict the response of *C. perfringens* in cooked meat during cooling, thereby enhancing risk assessment and therefore food safety with regard to *C. perfringens*.

A.2 Development of the Perfringens Predictor model

Perfringens Predictor predictions are based on a model developed at the Institute of Food Research. The purpose of this model is to predict the lag phase and growth response of *C. perfringens* in meat during cooling.

Unlike some earlier models which predominantly used the Gompertz sigmoid function, *Perfringens Predictor* uses the Baranyi model (Baranyi and Roberts, 1994). Because of the dynamic background of this model, predictions can be generated for fluctuating temperature profiles. In *Perfringens Predictor* the dynamic model is solved numerically, by the fourth-order Runge–Kutta method. The model is defined by four parameters:

- y_0 the natural logarithm of the initial cell concentration.
- μ_{max} the maximum specific growth rate (dynamically changing with temperature)
- y_{max} the natural logarithm of the maximum cell concentration.
- α_0 describes the 'initial physiological state' of the spores (see Baranyi and Roberts, 1994)

Perfringens Predictor predicts the net increase of *C. perfringens* in response to dynamic cooling conditions, considering a potential presence of *C. perfringens* spores at a concentration y_0. Predictions were performed, assuming a potential maximum 9 log increase of the bacterial concentration from the inoculum y_0.

The α_0 value is a dimensionless number between 0 and 1; if α_0=0, then there is no growth, and the lag time is infinite; if α_0=1, there is no lag, and growth will commence immediately. It has the role of an initial parameter, quantifying the history of the cells or spores. From that, the lag is a consequence, as the following formula in static conditions shows:

$$lag = -\frac{\ln(\alpha 0)}{\mu_{max}}$$

To solve the Baranyi model in fluctuating temperature, the temperature dependence of μ_{max}, the maximum specific growth rate must be modelled. Development of the growth rate model was carried out into two stages. 84 growth curves or growth rates were selected from the ComBase database (www.combase.cc) or produced from research carried out at the Institute of Food Research. Curves selected were obtained in meat or in culture medium:

- at static temperatures (ranging from to 15 to 52°C)
- for water activity values between 0.977 and 1
- for sodium nitrite concentrations between 0 and 150 ppm
- for pH values ranging from 5.2 to 8

For each growth curve, the maximum specific growth rate was estimated by fitting the model of Baranyi and Roberts (1994) to the experimental log counts.

Secondly, the following equation was fitted to the growth rates of *C. perfringens* calculated in the first step of the modelling:

$$\mu_{\max} = \begin{cases} b(T - T_{\min})^2 \left(1 - e^{c(T_{\max} - T)}\right)(aw - aw_{\min})(2 - aw - aw_{\min})(pH - pH_{\min}) \\ \qquad (NO_{2\max} - NO_2) \quad if \;\; pH < pH_s \\ b(T - T_{\min})^2 \left(1 - e^{c(T_{\max} - T)}\right)(aw - aw_{\min})(2 - aw - aw_{\min})(pH_s - pH_{\min}) \\ \qquad (NO_{2\max} - NO_2) \quad if \;\; pH \geq pH_s \end{cases} \tag{1}$$

where μ_{max} is the maximum specific growth rate and T, pH, and NO_2, the temperature, pH and water acitivity respectively. *b*, *c*, T_{min} (theoretical minimum temperature for growth), T_{max} (maximum temperature for growth), pH_{min} (minimum pH for growth), aw_{min} (minimum aw for growth), NO_{2max} (maximum pH for growth) and pH_s (a threshold value above which μ_{max} does not change) are the parameters of the model. Before fitting, a square root transformation was performed to homogenize the variance of the growth rate. Estimated parameter values are provided in Table 1.

Table 1. Fitted parameters of the growth rate model

Parameter	Estimate	Standard error
b	0.03901	0.00866
T_{min}	12.20	0.87
c	0.0947	0.0252
T_{max}	54.47	0.52
pH_{min}	4.76	0.13
pH_s	6.25	0.05
aw_{min}	0.9755	0.0006
NO_{2min}	191	15

Lag is more difficult to model than the maximum specific growth rate as it depends not only on the current environmental conditions but also on the history (physiological state) of the spores. Spores that are damaged after a heat treatment may require more time to germinate and/or repair damage before growth commences. As previously mentioned, the Baranyi model describes the lag time through an α_0 value (a dimensionless number between 0 and 1) related to the physiological sate of the spores.

Perfringens Predictor has been developed to predict the growth of *C. perfringens* during cooling, after a heating process that is may be damaging for the spores. Therefore it is important to determine which α_0 values should be used to reflect the physiological state of *C. perfringens* after typical heating profiles which may be applied by the food industry.

Experiments carried out at the Institute of Food Research (maximum temperature in the range of 70-95°C) suggest that in cured products, a α_0 value of 0.001 should be used. However, in uncured products, an α_0 value of 0.001 should be used for NaCl concentrations less than 1% and a value of 0.01 for NaCl concentrations higher or equal to 1%. Perfingens Predictor automatically selects the appropriate value.

A.3 Validation of Perfringens Predictor

Perfringens Predictor has been validated for use with different meats. Examples from IFR of the observed kinetics of *C. perfringens* during meat cooling, and prediction obtained from Perfringens Predictor are illustrated in Figures 6 to 25. The meats used in these tests were sterile beef, pork, or turkey slurry, with the pH adjusted to values between 5.5 and 6.3, and NaCl concentration between 0.5 and 3.0%. $NaNO_2$ was added to some of the sterile pork slurries to give input concentrations between 10 and 60 ppm. Further examples from IFR of the observed kinetics of *C. perfringens* during meat cooling and prediction obtained from Perfringens Predictor are illustrated in Figures 26 to 30. The meats used in these tests were either sterile beef or sterile turkey slurry, with no added preservatives.

Examples of comparisons of observed kinetics of *C. perfringens* during meat cooling from the literature, and the prediction obtained from Perfringens Predictor based on the cooling curves are shown in Figures 31 to 43 and Table 2. Details of the actual meats are given in the Figure and Table legends.

Predictions from Perfringens Predictor are based on (i) the heat treatment being in the range of 70°C (for up to 6 hours) to 95°C (for up to 1.5 hours). If lower heat treatments are used, growth of *C. perfringens* might be faster than predicted by the model, while if higher heat treatments are used, growth of *C. perfringens* might be slower than predicted by Perfringens Predictor. The pH of the meat should be between 5.2 and 8.0, and NaCl concentration 0 to 4% (or water activity 0.977 to 1).

Key to Figures 6-25: Red dashed line represents the temperature profile; the solid blue line represents the predicted increase in viable count based on the cooling profile and pH/NaCl/$NaNO_2$; and the blue squares represent the viable counts. The meats used in these tests were sterile beef, pork, or turkey slurry. $NaNO_2$ (sodium nitrite) was added immediately prior to heating.

Figure 6. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.3, 0.5% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

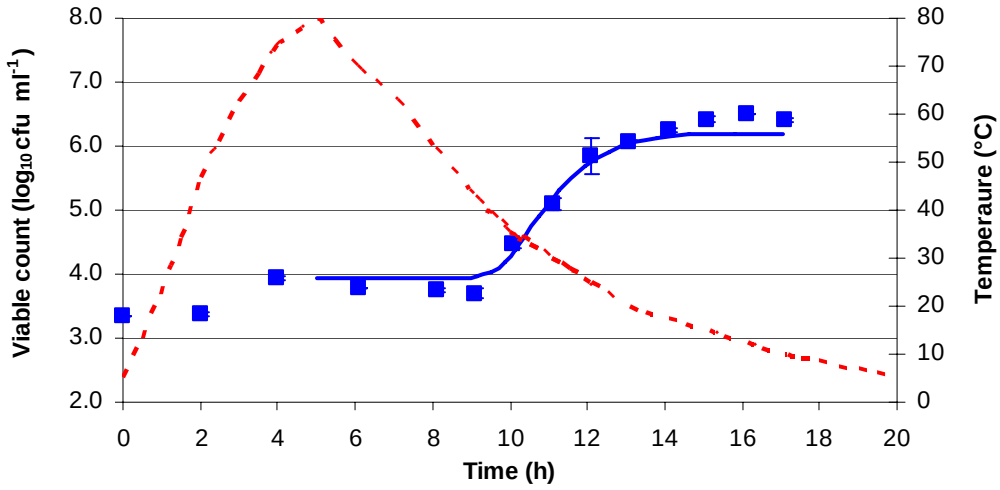


Figure 7. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 1.5% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

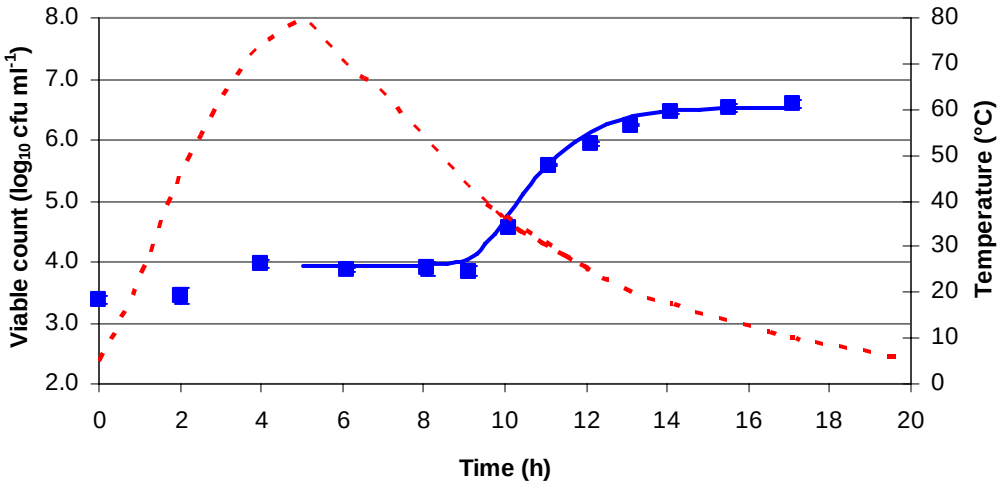

Viable count (log10 cfu ml-1)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
Temperature (°C)
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Time (h)

Figure 8. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 2.5% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

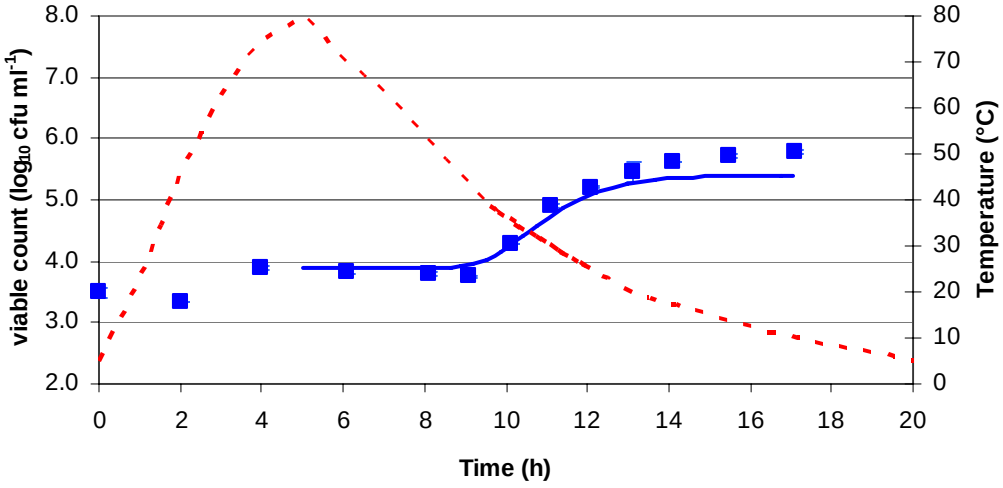


Figure 9. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 3.0% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

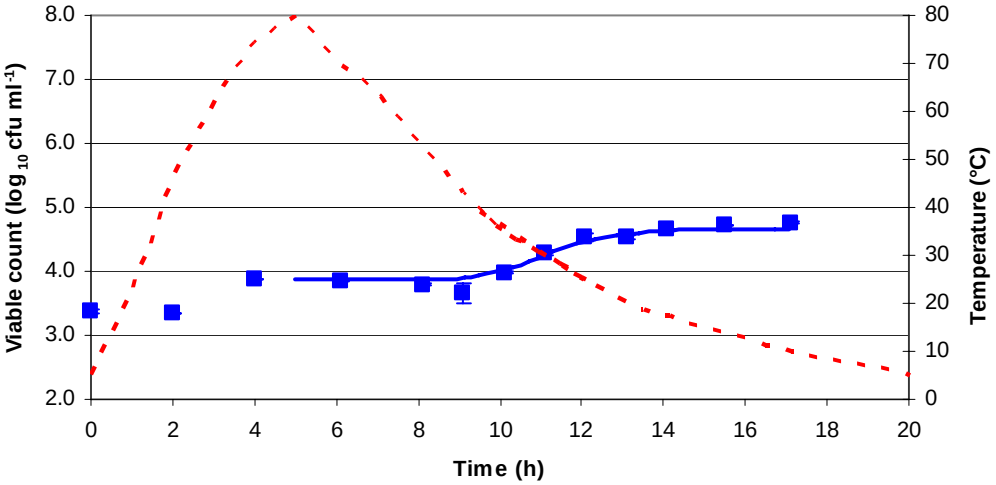


Figure 10. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 5.5, 0.5% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

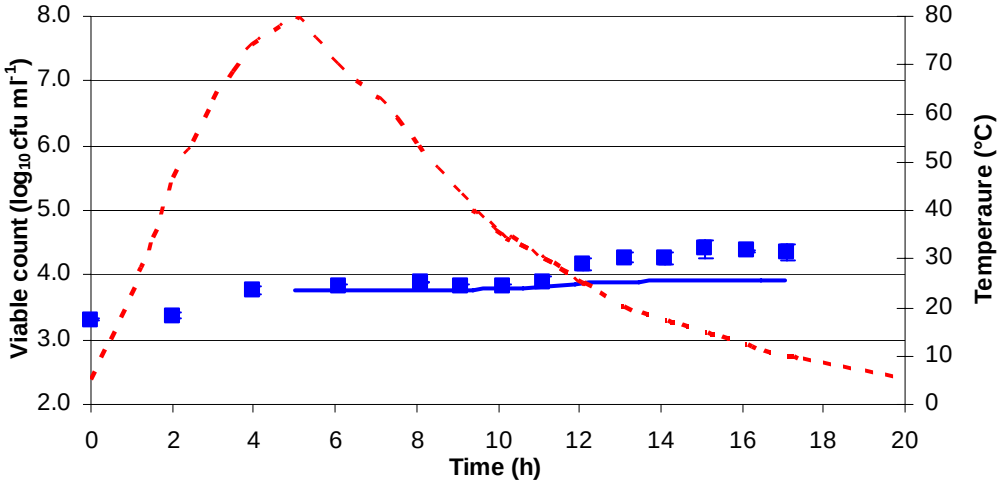


Figure 11. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 5.5, 3.0% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

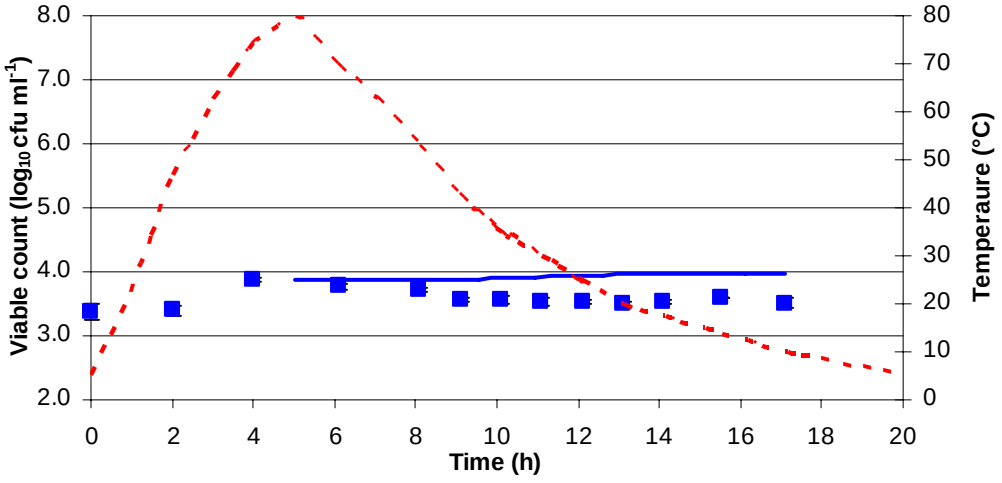


Figure 12. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 0.5% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C).

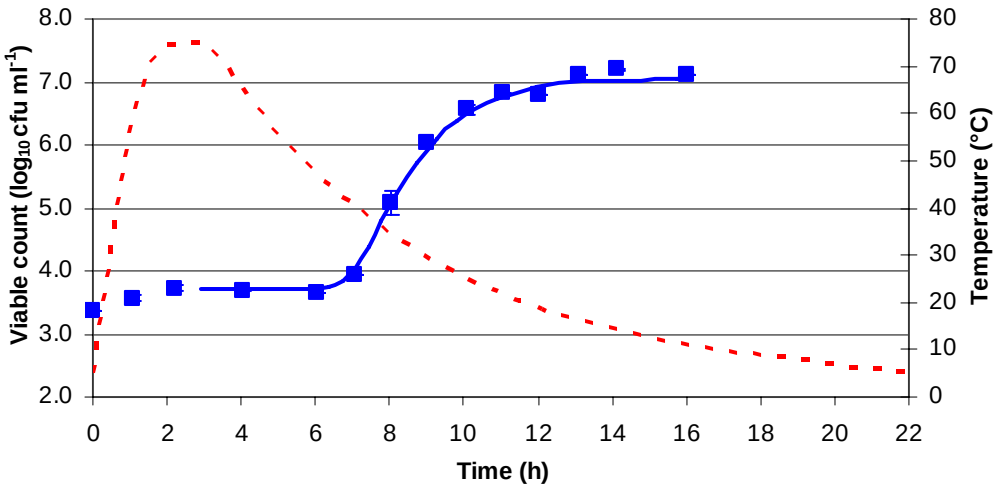


Figure 13. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 1.5% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C).

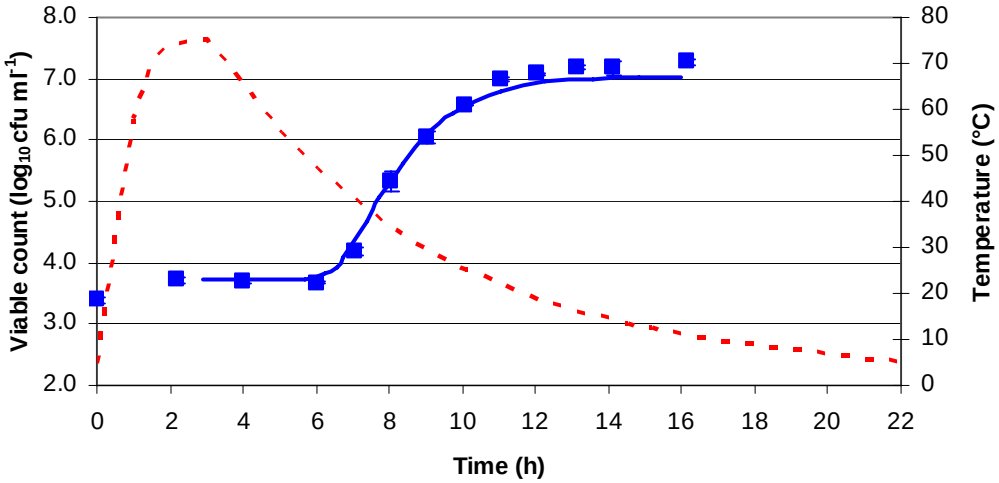


Figure 14. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 2.5% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C).

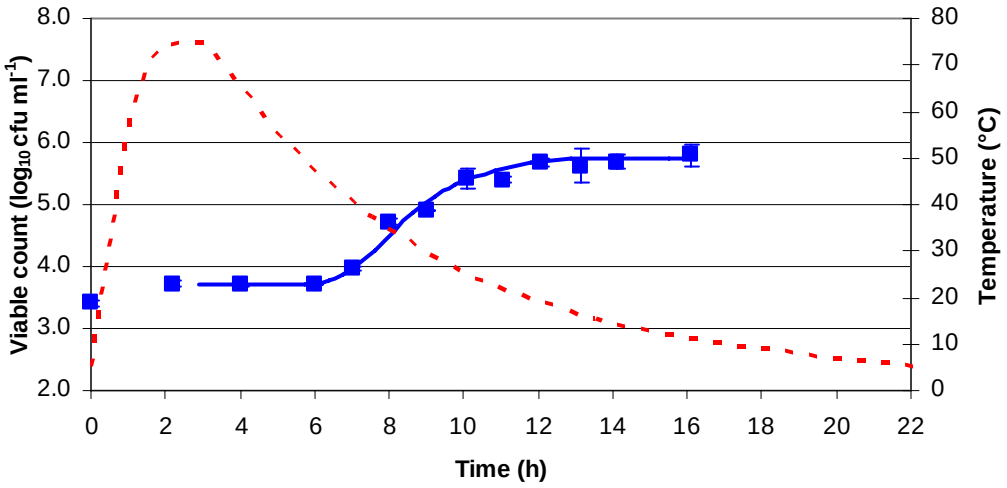


Figure 15. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 3.0% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

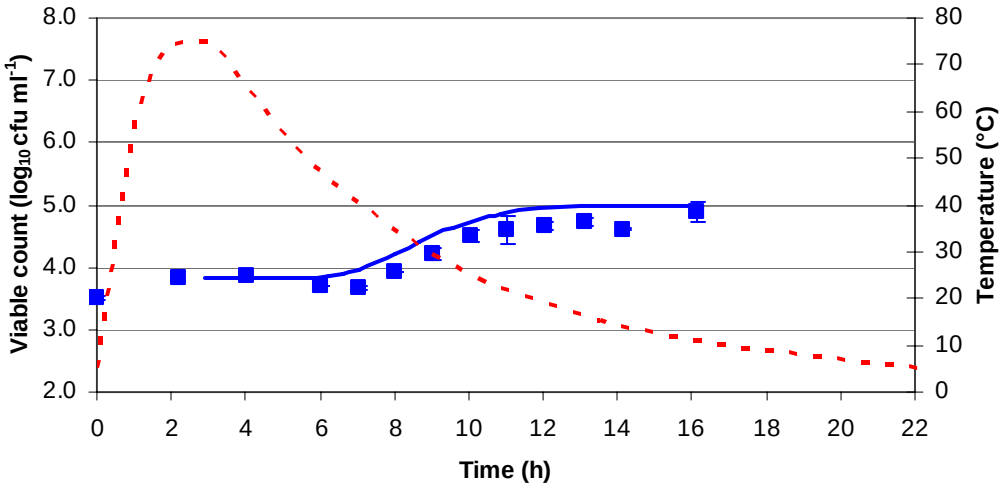


Figure 16. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 3.0% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

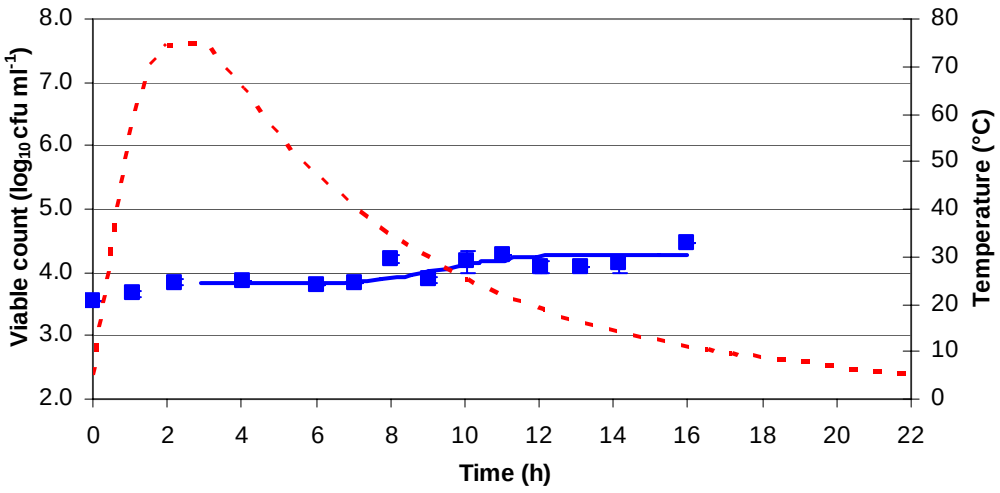


Figure 17. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 3.0% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

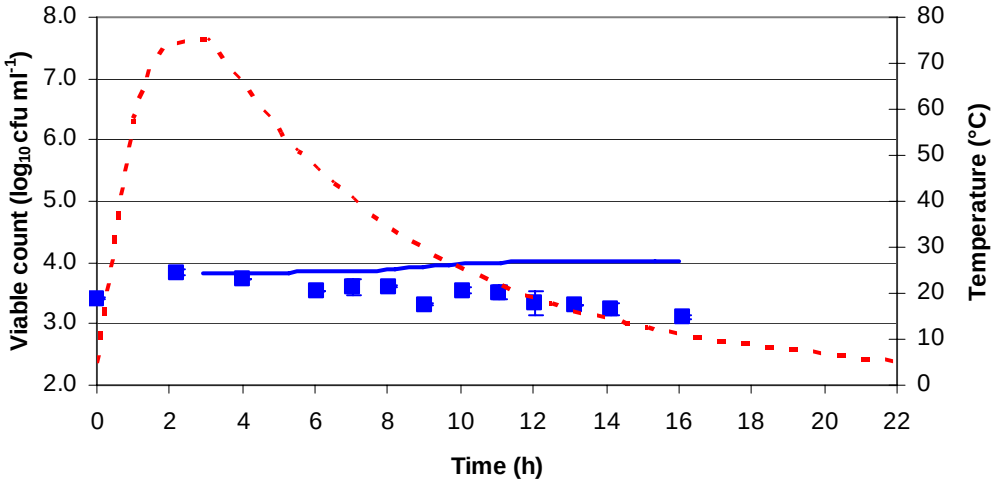


Figure 18. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 5.6, 1.0% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

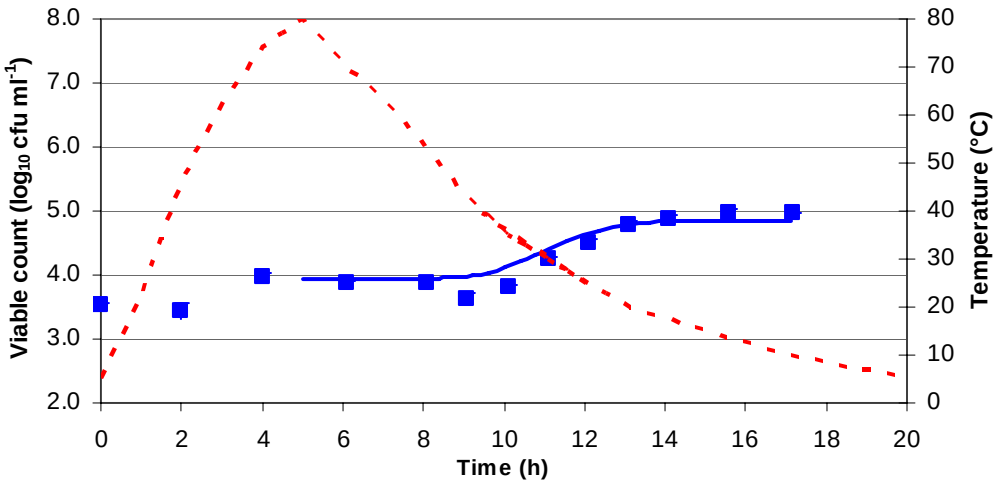


Figure 19. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 5.7, 2.0% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

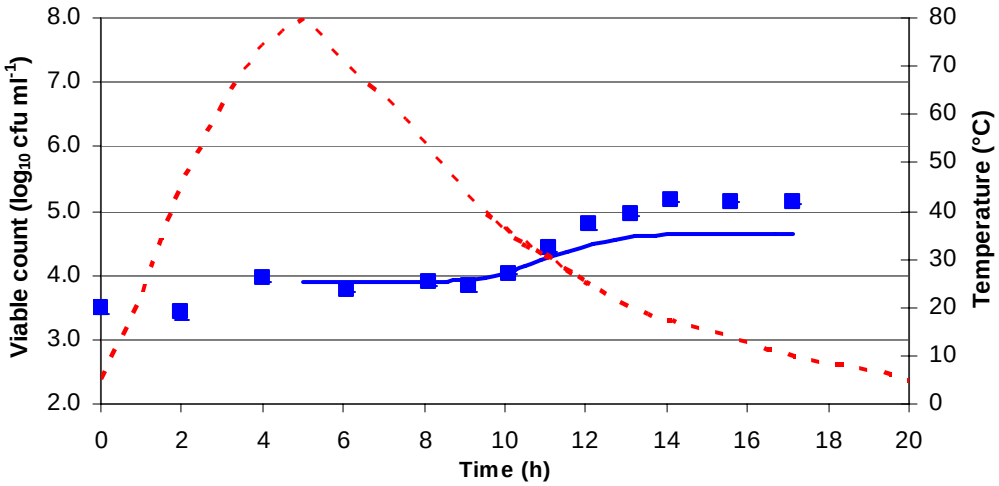


Figure 20. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.1, 1.5% NaCl, 10 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

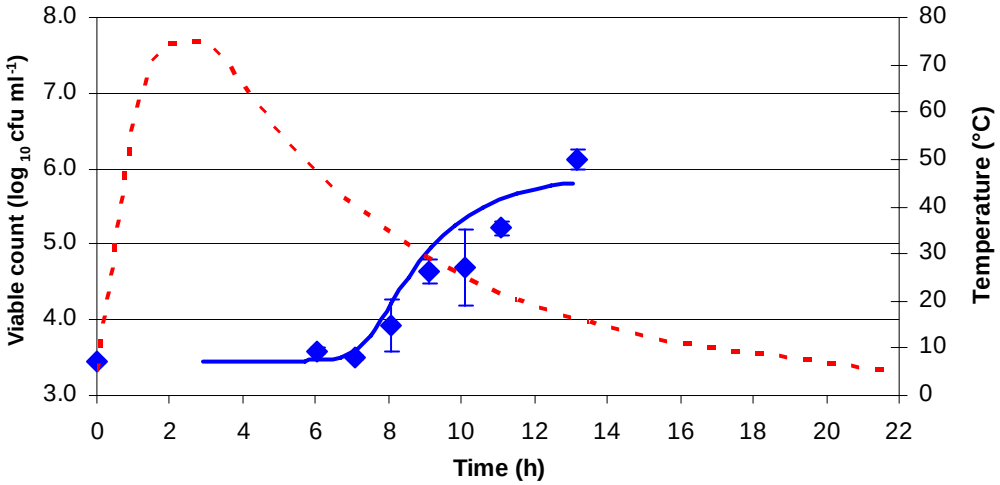


Figure 21. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.1, 1.5% NaCl, 15 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

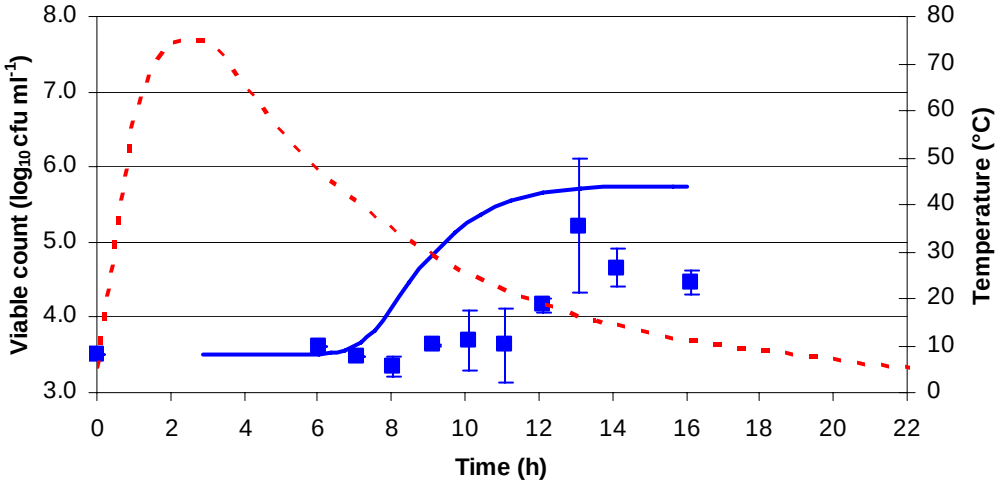


Figure 22. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.1, 1.5% NaCl, 60 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

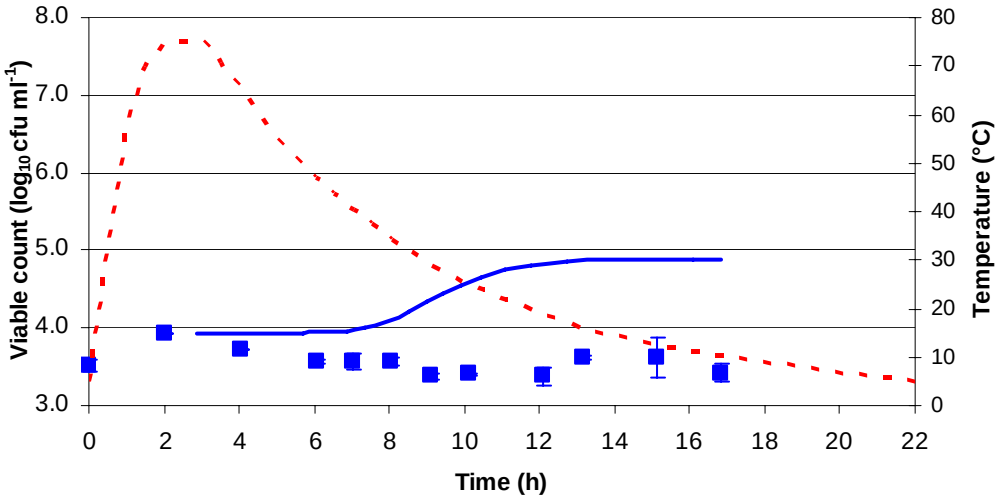


Figure 23. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.2, 2.5% NaCl, 10 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

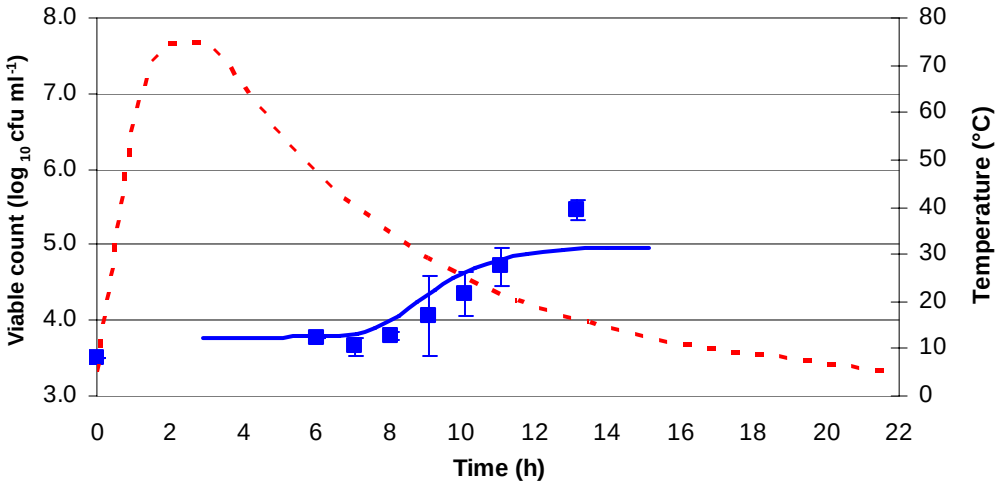


Figure 24. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.2, 2.5% NaCl, 15 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

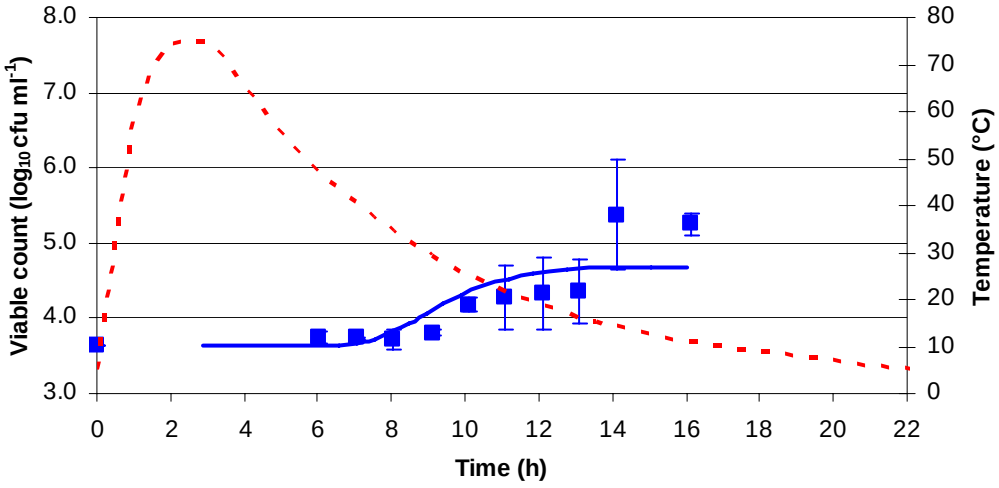
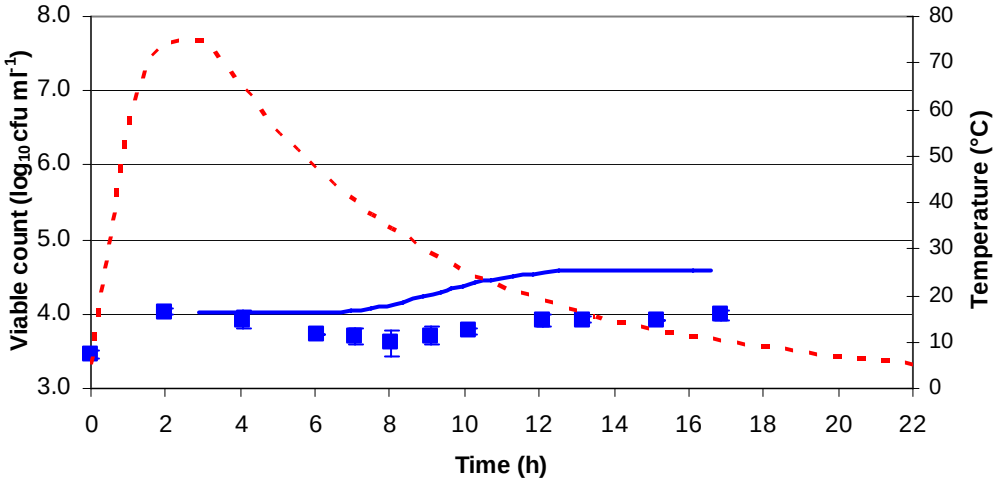


Figure 25. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.2, 2.5% NaCl, 40 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)



Key to Figures 26-30: Red dashed lines represent dynamic cooling profiles; each of the solid green, blue or black profiles represent predictions for different starting concentrations of spores for respective heat treatments; and points represent experimental observations for those respective spore concentrations. The meats used in these tests were either sterile beef or sterile turkey, with no preservatives added.

Figure 26. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C).

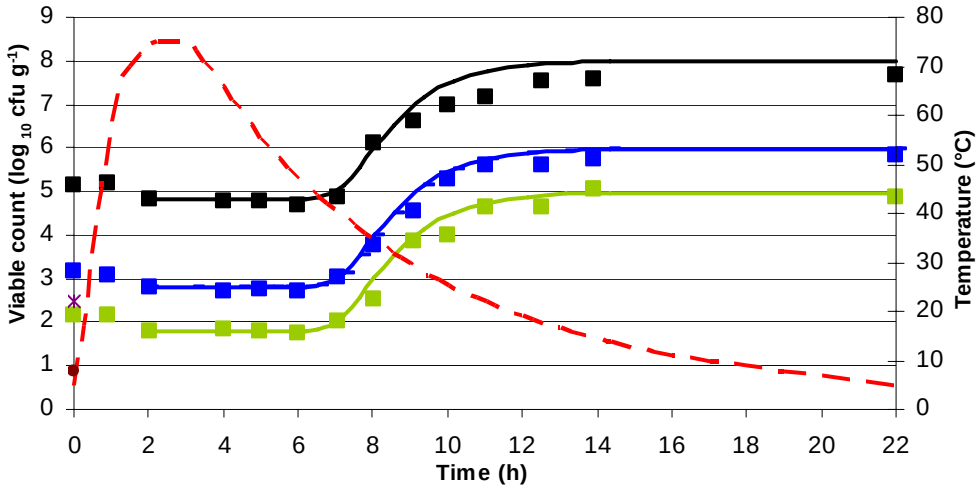


Figure 27. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

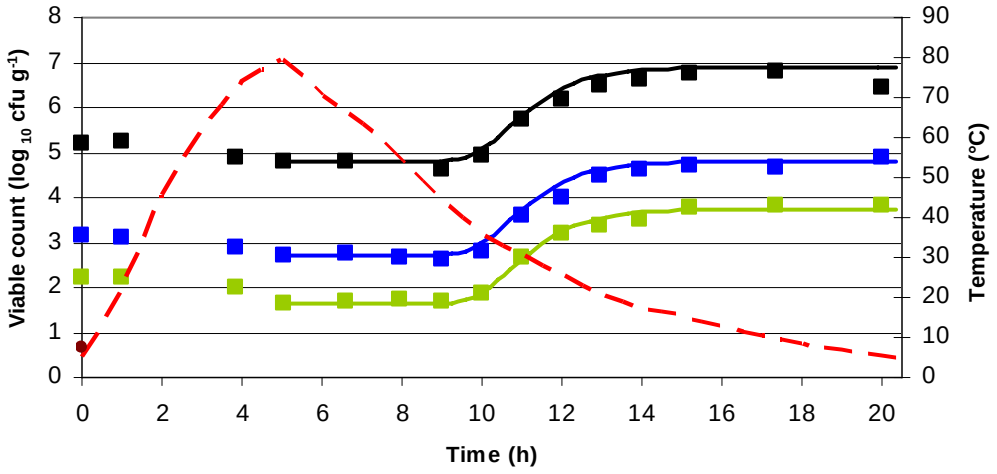


Figure 28. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), after 3h heat-up from 5°C to 75°C, followed by 7 h exponential cooling to 5°C).

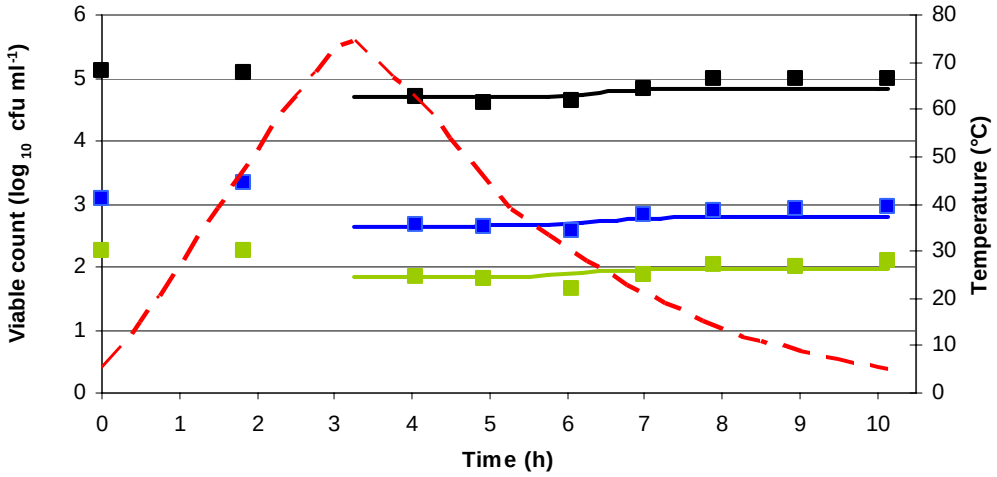


Figure 29. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), after 2h heat-up from 5°C to 95°C, 95°C/1h followed by 19h exponential cooling to 5°C).

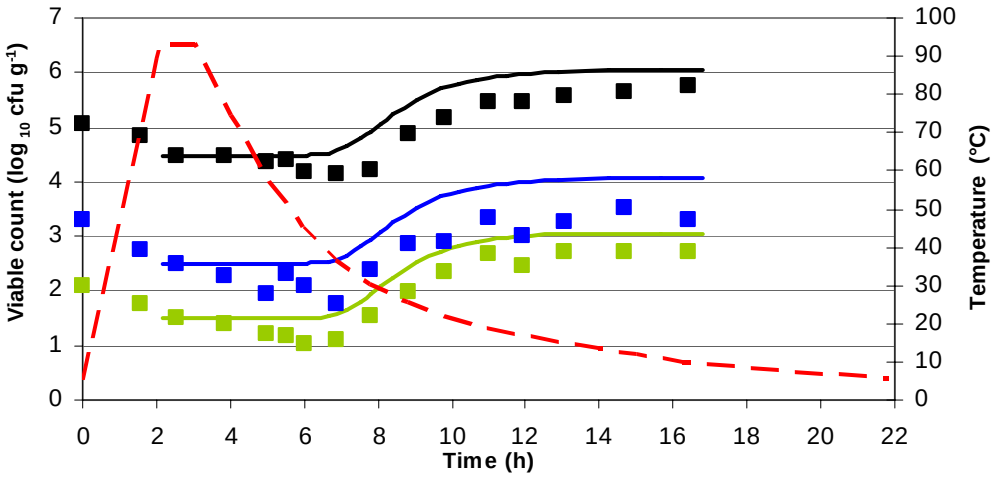
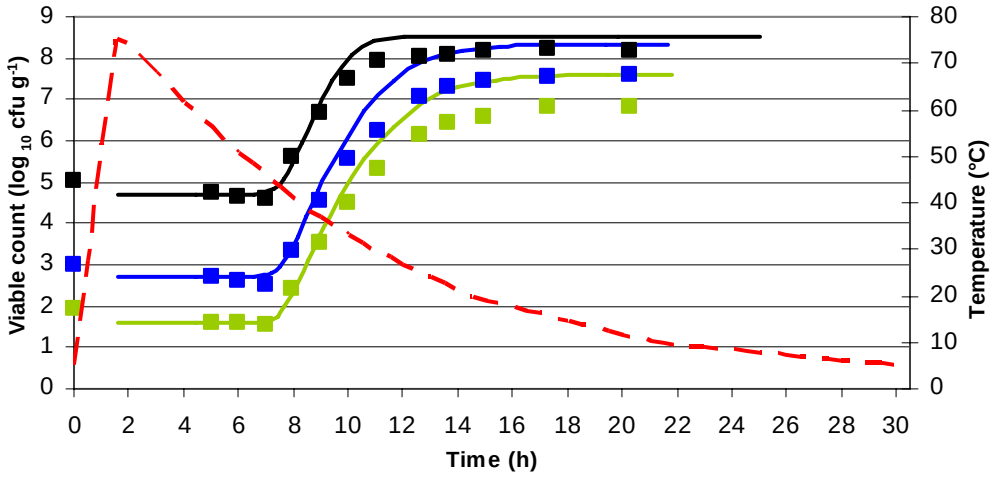


Figure 30. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), after 2h heat-up from 5°C to 75°C followed by 28h exponential cooling to 5°C).



Figures 31 to 43 show examples of comparisons of observed kinetics of *C. perfringens* during meat cooling from the literature, and prediction obtained by Perfringens Predictor. Details of the actual meats are given in the figure legends.

Key to Figures 31-43: Red dashed lines represent dynamic cooling profiles, the solid black profiles represents the prediction from Perfringens Predictor, points represent experimental observations reported by the original authors.

Figure 31. Comparison of output from Perfringens Predictor with observations of Smith *et al.* [2004]. (The experimental system was ground beef, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation) with cooling from 54.4°C to 4.4°C in 21 hours).

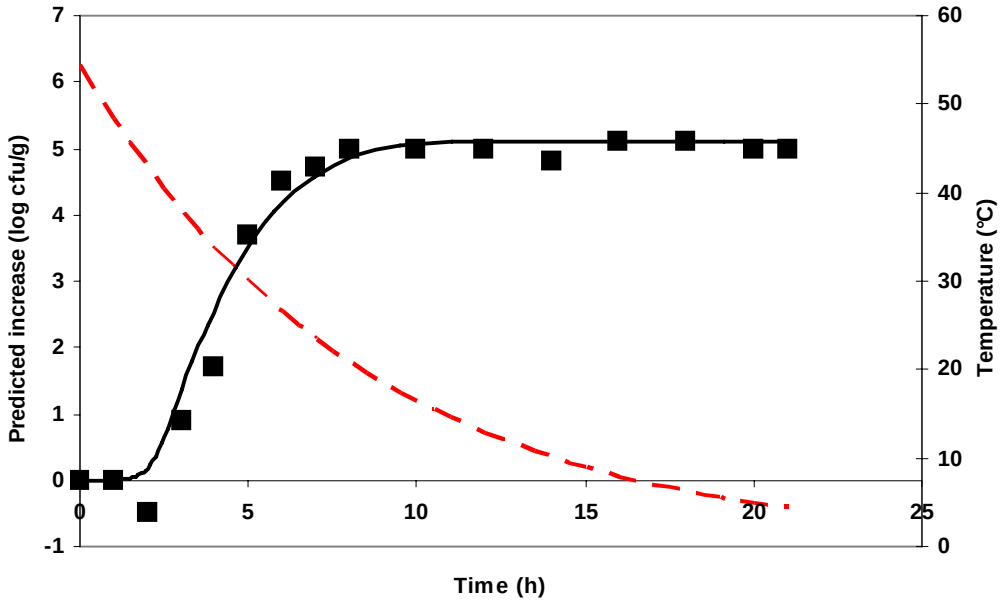


Figure 32. Comparison of output from Perfringens Predictor with observations of Smith and Schaffner [2004]. (The experimental system was broth, and the spores were heated at 75°C for 20 min (for activation)).

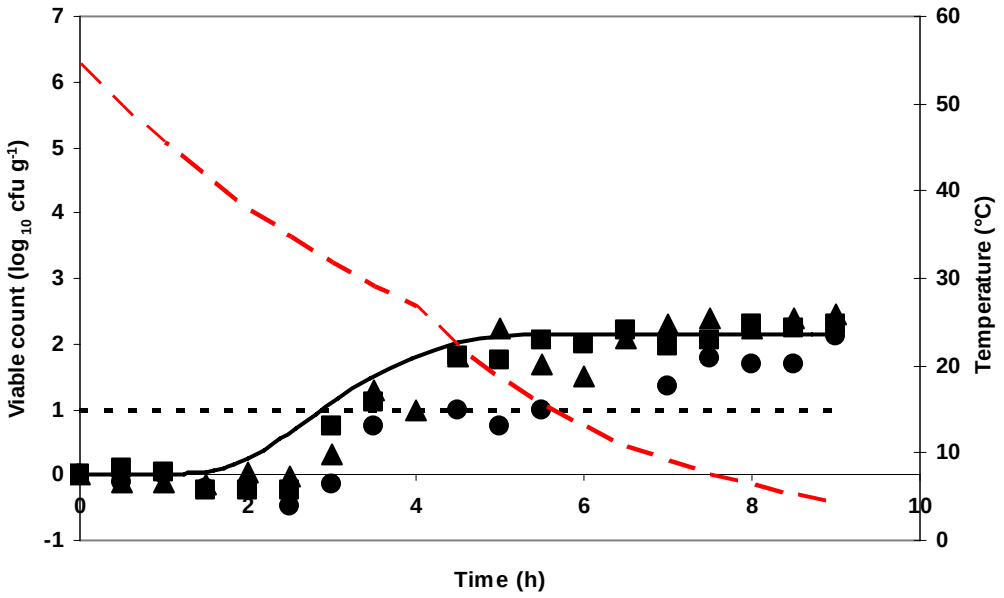


Figure 33. Comparison of output from Perfringens Predictor with observations of Blankenship *et al* [1988]. The experimental system was cooked chilli, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation)).

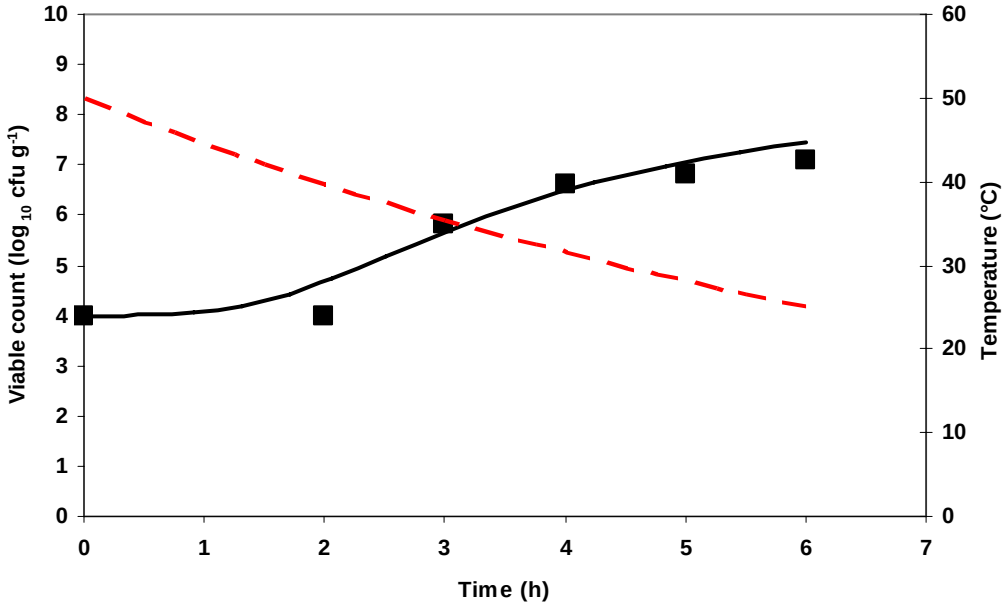


Figure 34. Comparison of output from Perfringens Predictor with observations of Shigehisa *et al.* [1985]. (The experimental system was heat treated mince turkey, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 80°C for 15 min (for activation)).

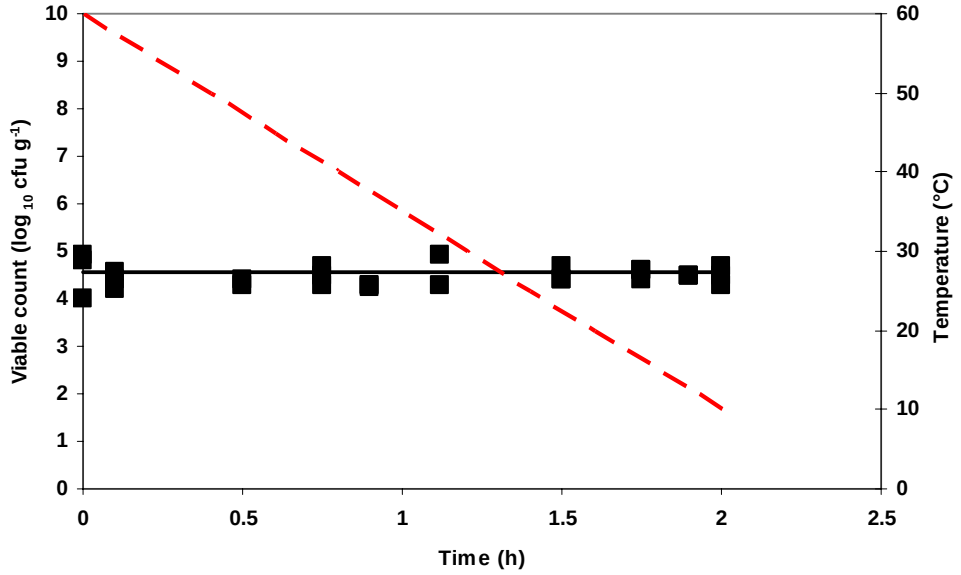


Figure 35. Comparison of output from Perfringens Predictor with observations of Shigehisa *et al.* [1985]. (The experimental system was heat treated mince turkey, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 80°C for 15 min (for activation)).

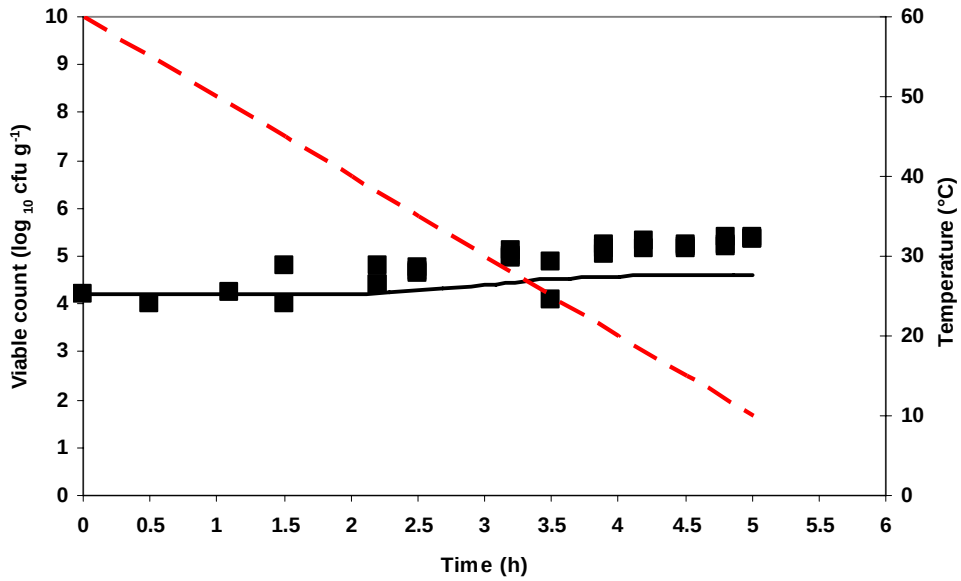


Figure 36. Comparison of output from Perfringens Predictor with observations of Shigehisa *et al.* [1985]. (The experimental system was heat treated mince turkey, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 80°C for 15 min (for activation)).

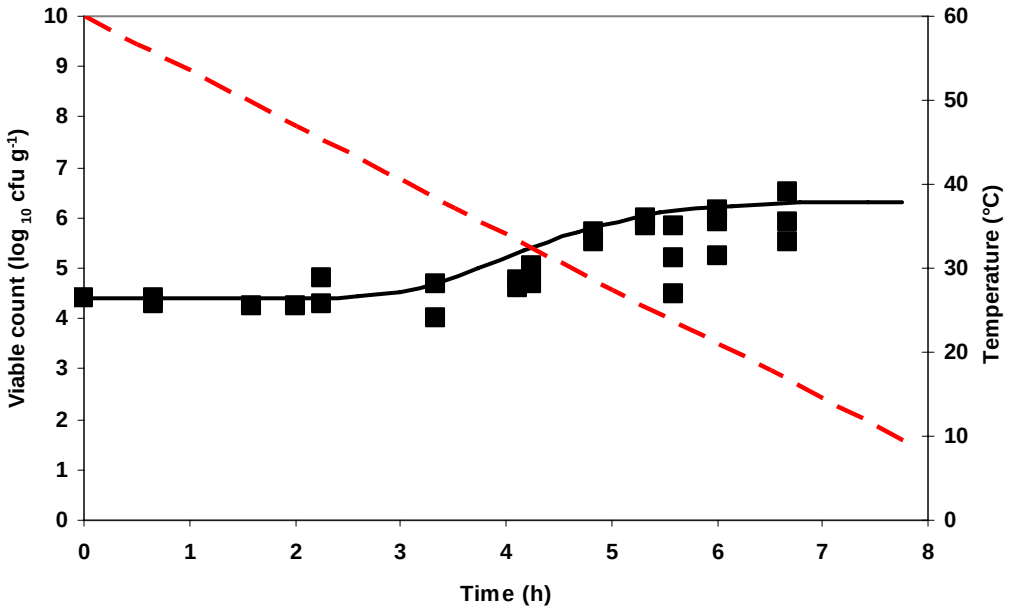


Figure 37. Comparison of output from Perfringens Predictor with observations of Shigehisa *et al.* [1985]. (The experimental system was heat treated mince turkey, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 80°C for 15 min (for activation)).

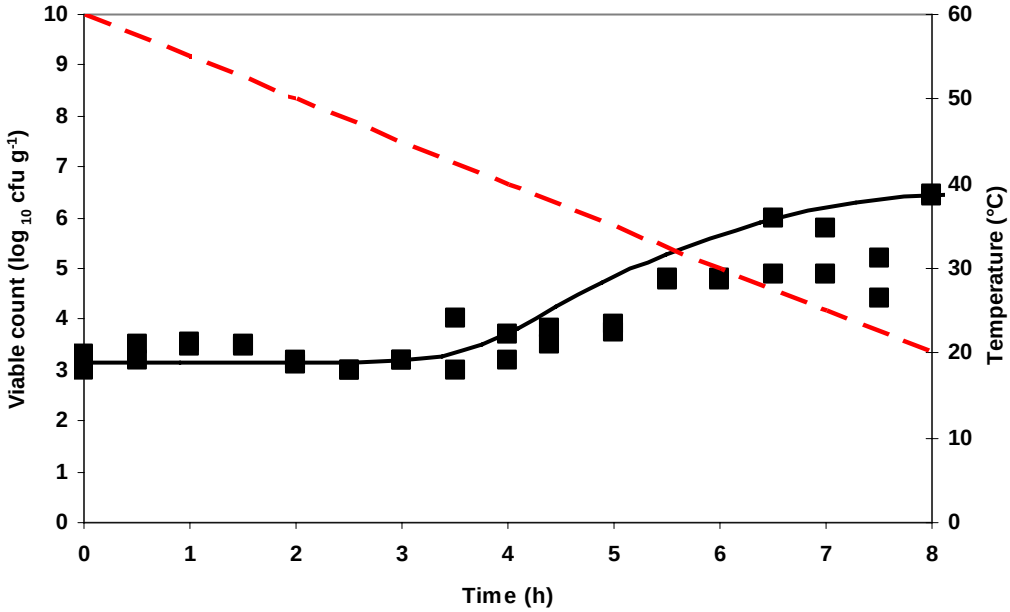


Figure 38. Comparison of output from Perfringens Predictor with observations of Sanchez *et al.* [2005]. (The experimental system was heat treated mince beef, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation)).

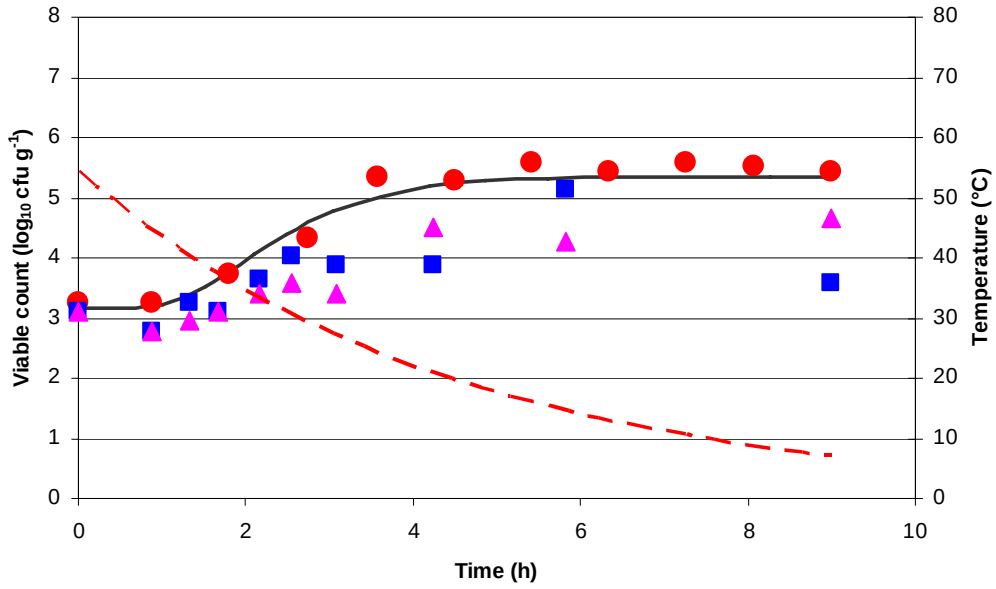


Figure 39. Comparison of output from Perfringens Predictor with observations of Sanchez *et al.* [2005]. (The experimental system was heat treated mince beef, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation)).

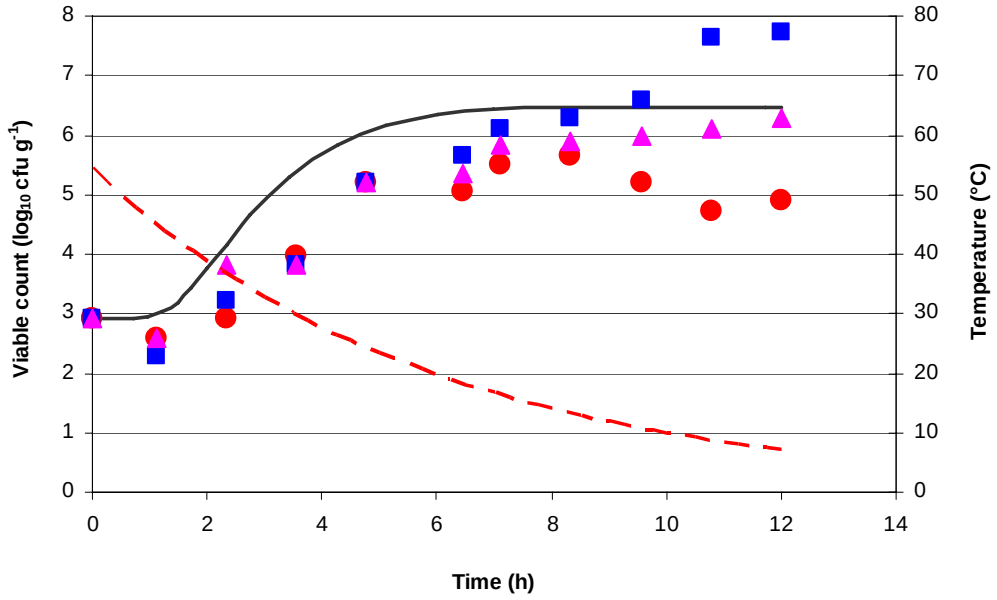


Figure 40. Comparison of output from Perfringens Predictor with observations of Sanchez *et al.* [2005]. (The experimental system was heat treated mince beef, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation)).

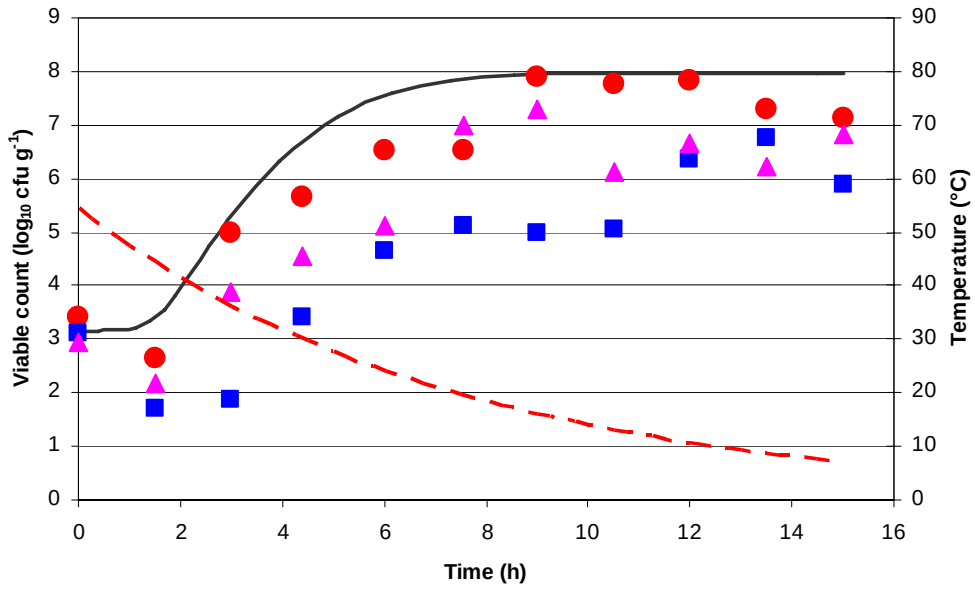


Figure 41. Comparison of output from Perfringens Predictor with observations of Sanchez *et al.* [2005]. (The experimental system was heat treated mince beef, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation)).

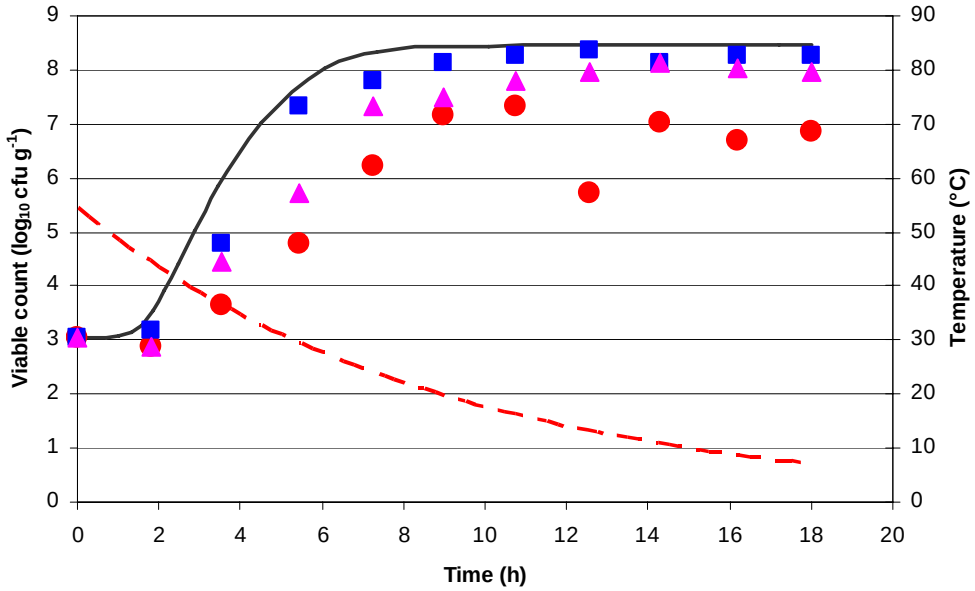


Figure 42. Comparison of output from Perfringens Predictor with observations of Amezquita *et al.* [2005]. The experimental system was cured ham pH 6.1, containing 50 ppm $NaNO_2$ (estimated to be the concentration at the start of cooling) and 1.25% NaCl. The spores were heated at 75°C for 20 min (for activation).

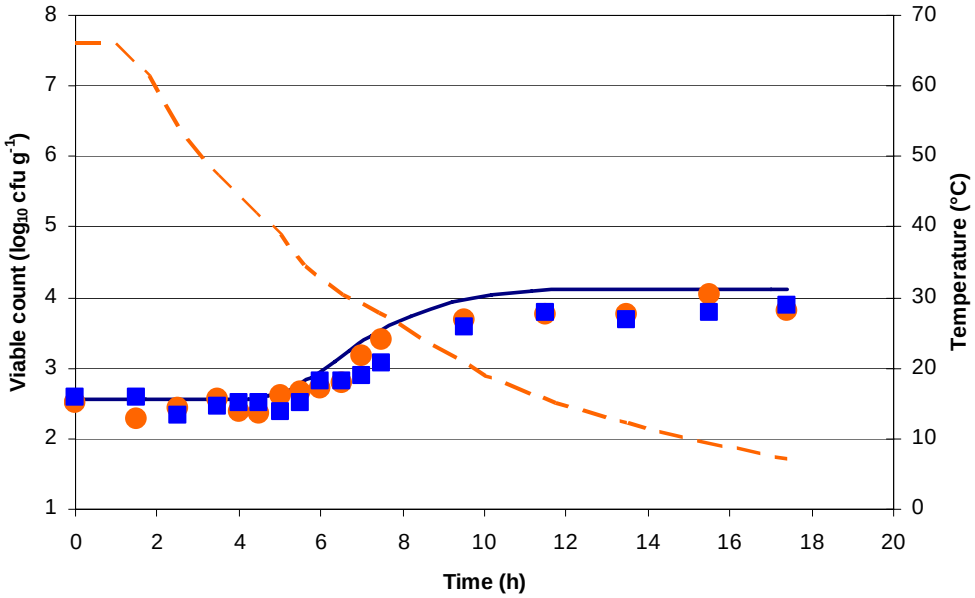


Figure 43. Comparison of output from Perfringens Predictor with observations of Amezquita *et al.* [2005]. The experimental system was cured ham pH 6.1, containing 50 ppm $NaNO_2$ (estimated to be the concentration at the start of cooling) and 1.25% NaCl. The spores were heated at 75°C for 20 min (for activation).

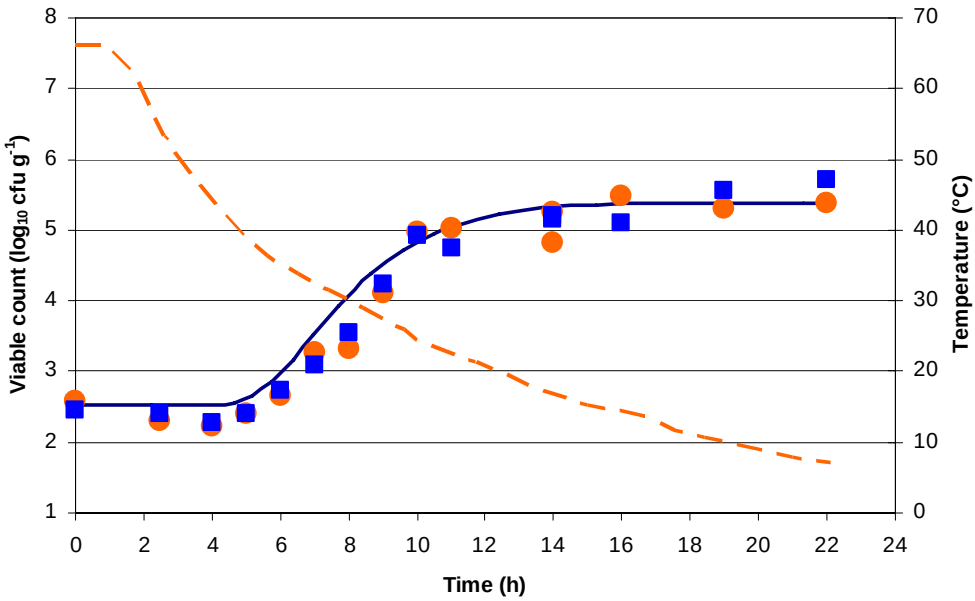


Table 2. Comparison of output from Perfringens Predictor with observations of Thippareddi *et al.* [2003]. The experimental system was roast beef (pH 5.6) or injected pork (pH 6.0), 0.5% NaCl, $NaNO_2$ (0 ppm). The spores were heated at 75°C for 20 min (for activation).

Meat	Cooling time	Observed log increase		Predicted log increase
		Mean	Maximum	
Roast beef	18 h 21 h	1.5 5.3	3.5 5.4	2.0 3.0
Injected pork	18 h 21 h	3.7 4.4	4.5 5.1	3.4 4.8

A.4 References:

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