ComBase Predictor

User Manual

Version 3.0

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Section 1: Introduction to *ComBase Predictor 3.0*

1.1 Basic predictive microbiology

Predictive microbiology is the science of describing and predicting, by mathematical means, the response of microorganisms to their environments. If responses are observed under a sufficient number of combinations of the environmental factors, then it is possible to predict how the organism would respond to any conditions in the environmental region where the observations were made. If, for example, you know how a microbe will grow, survive or die at particular combinations of temperature and pH, then it is possible to use a set of mathematical equations to predict how it will grow, survive or die when conditions are changed.

1.2 Why might predictive microbiology be useful to me?

If you are involved in product development, then predictive microbiology techniques will enable you to judge the effect of changing product formulations on the possible growth of pathogens or spoilage organisms; this could considerably reduce the amount of testing of intermediate or prototype products that is necessary. However, note that use of such predictions is not a substitute for exhaustive testing of a final product formulation before release.

1.3 What is *ComBase Predictor*?

ComBase Predictor comprises a set of new models predicting the response of the organisms as a function of the environmental factors, including temperature, pH and water activity. Some models also include an additional, fourth factor, such as the concentration of carbon dioxide or acetic acid. Survival and death models are also included in *ComBase Predictor*.

ComBase Predictor has a number of notable features:

- it can be used to make predictions in either a static temperature or under fluctuating (changing) temperature conditions
- it can provide up to four simultaneous predictions
- it includes growth and survival curves as well as thermal and non-thermal death curves

1.4 *ComBase Predictor* overview

In the first version of *ComBase Predictor*, the models were based on the data used to develop the previous UK predictive modelling software, Food MicroModel. In version 2.0, these datasets were expanded with relevant growth/death curves from the predictive microbiology database Combase (see www.combase.cc). Those logcount growth and survival curves were considered that enabled us to estimate the exponential growth/death rates with high confience. The inclusion of new datasets allowed us to expand the range of environmental factors where predictions could be generated.
Furthermore, new models have been developed for microorganisms for which predictions were unavailable in the previous version of *ComBase Predictor*. Table

1 and 2 lists the models currently available and indicates which models have been created or updated.

More details on the data sets and on the mathematical and modelling aspects of *ComBase Predictor 2.0* are provided in Section 3 and in the Appendix.

Version 3.0 has improved the layout and

Table 1. Summary of raw datasets (observed "logcount v. time" curves) selected to develop growth models. Initial datasets: observed growth curves used to generate the growth models in the previous version of ComBase Predictor.

		(3 factors*) (4th		factor**)	
Growth	**Extended**	**No. of curves in the initial set**	**No. of newly included curves**	**No. of curves in the initial set**	**No. of newly included curves**
Aeromonas hydrophila	**Yes**	125	**33**	-	**-**
Bacillus cereus with CO2(%)	**Yes**	86	**109**	58	**-**
Bacillus licheniformis	No 53		**-**	-	**-**
Bacillus subtilis	No 68		**-**	-	**-**
Clostridium botulinum (non-prot.)	No 52		**-**	-	**-**
Clostridium botulinum (prot.)	No 77		**-**	-	**-**
Clostridium perfringens	No 51		**-**	-	**-**
Escherichia coli with CO2(%)	**Yes**	80	**89**	78	**-**
Listeria monocytogenes / innocua with CO2(%)	**Yes**	251	**279**	75	**9**
Listeria monocytogenes / innocua with nitrite(ppm)	**Yes**	251	**279**	63	**118**
Listeria monocytogenes / innocua with lactic(ppm)	**Yes**	251	**279**	129	**-**
Listeria monocytogenes / innocua with acetic(ppm)	**Yes**	251	**279**	52	**-**
Staphylococcus aureus	No 93		**-**	-	**-**
salmonellae with CO2(%)	**Yes**	146	**113**	23	**-**
salmonellae with nitrite(ppm)	**Yes**	146	**113**	28	**-**
Shigella flexneri with nitrite (ppm)	**Yes**	-	**193**	-	**125**
Yersinia enterocolitica with CO2(%)	**Yes**	282	**35**	31	**-**

Yersinia enterocolitica with lactic(ppm)	**Yes**	282	**31**	77	**-**
Brochothrix thermosphacta	No 44		**-**	-	**-**
Pseudomonas spp	**Yes**	-	**144**	-	**-**

* 3 factors: temperature, pH and aw
** 4th factor: additional facor (either CO_2 or lactic acid or acetic acid or nitrite)

Table 2. Summary of raw datasets (observed "logcount v. time" curves) selected to develop thermal inactivation models. Initial datasets: observed growth curves used to generate the growth models in the previous version of ComBase Predictor.

		(3 factors*) (4th		factor**)	
Thermal inactivation	**Extended**	**No. of curves in the initial set**	**No. of newly included curves**	**No. of curves in the initial set**	**No. of newly included curves**
Bacillus cereus	No	41 - - -			
Clostridium botulinum (non-prot.)	No 35	- - -			
Escherichia coli	**Yes**	51	**21**	- -	
Listeria monocytogenes/innocua	**Yes**	67	**149**	- -	
Salmonellae	**Yes**	61	**8**	- -	
Yersinia enterocolitica	**Yes**	82	**4**	- -	
Brochothrix thermosphacta	**Yes**	-	**53**	- -	

* 3 factors: temperature, pH and aw
** 4th factor: additional factor (either CO_2 or lactic acid or acetic acid or nitrite)

Table 3. Summary of raw datasets (observed "logcount v. time" curves) used to develop non-thermal survival models.

NEW non-thermal survival models	**No. of observed survival curves selected**
Listeria monocytogenes/innocua	**194**
salmonellae	**102**

* 3 factors: temperature, pH and aw
** 4th factor: additional factor (either CO_2, lactic acid, acetic acid or nitrite)

Section 2: How to use *ComBase Predictor*

2.1 Registration

Before using *ComBase Predictor*, you need to register

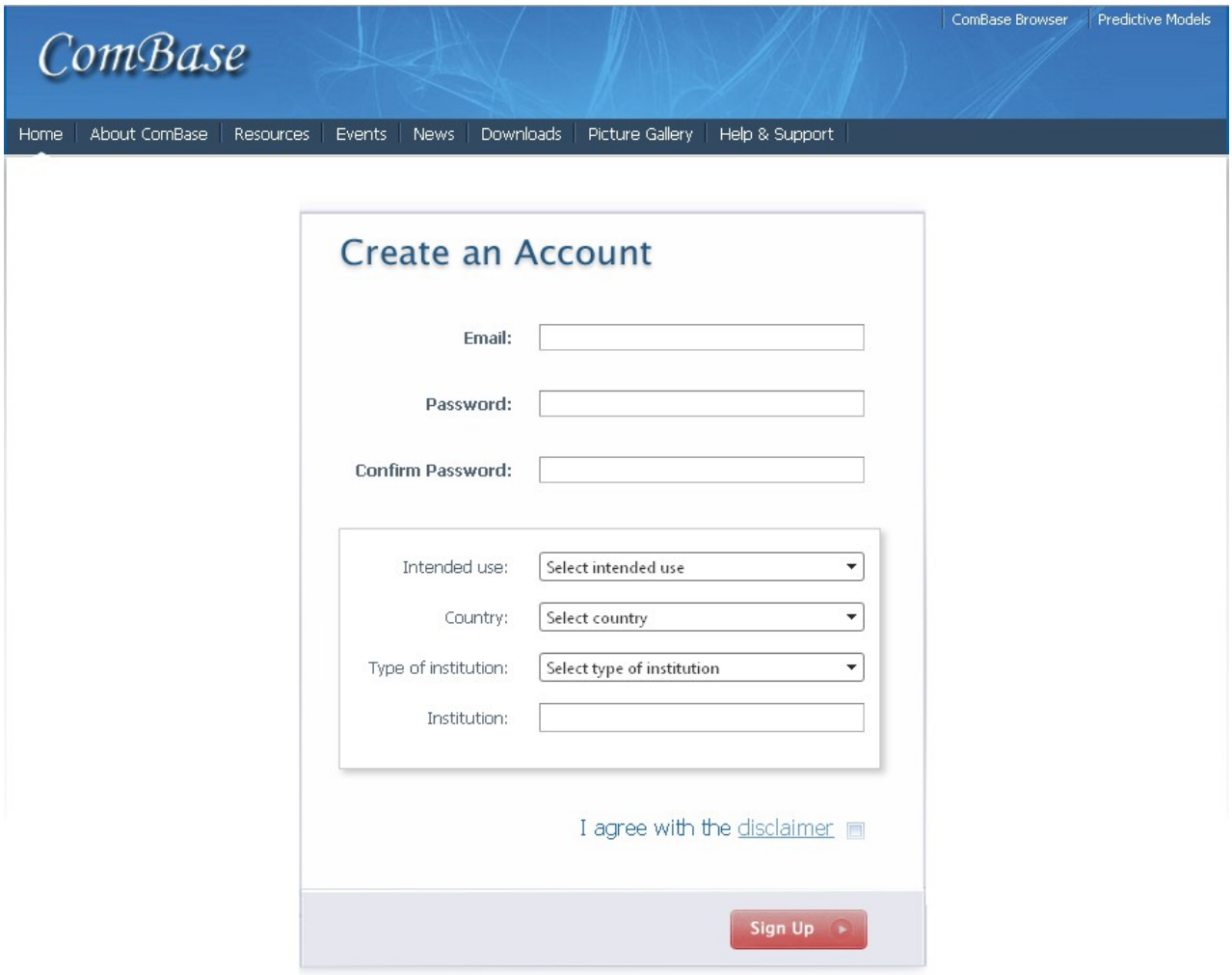
Your registration details will not be passed to third parties

Your email address will be added to our own mailing list so that we can provide you with occasional email alerts on updates and major ComBase-related events. If you wish to opt out the mailing list, you can unsubscribe at any time modifying your User Account Settings

To register to, follow the instructions from the *ComBase Predictive Models* menu point in the www.combase.cc web page.

A screen shot of the Register form is shown below

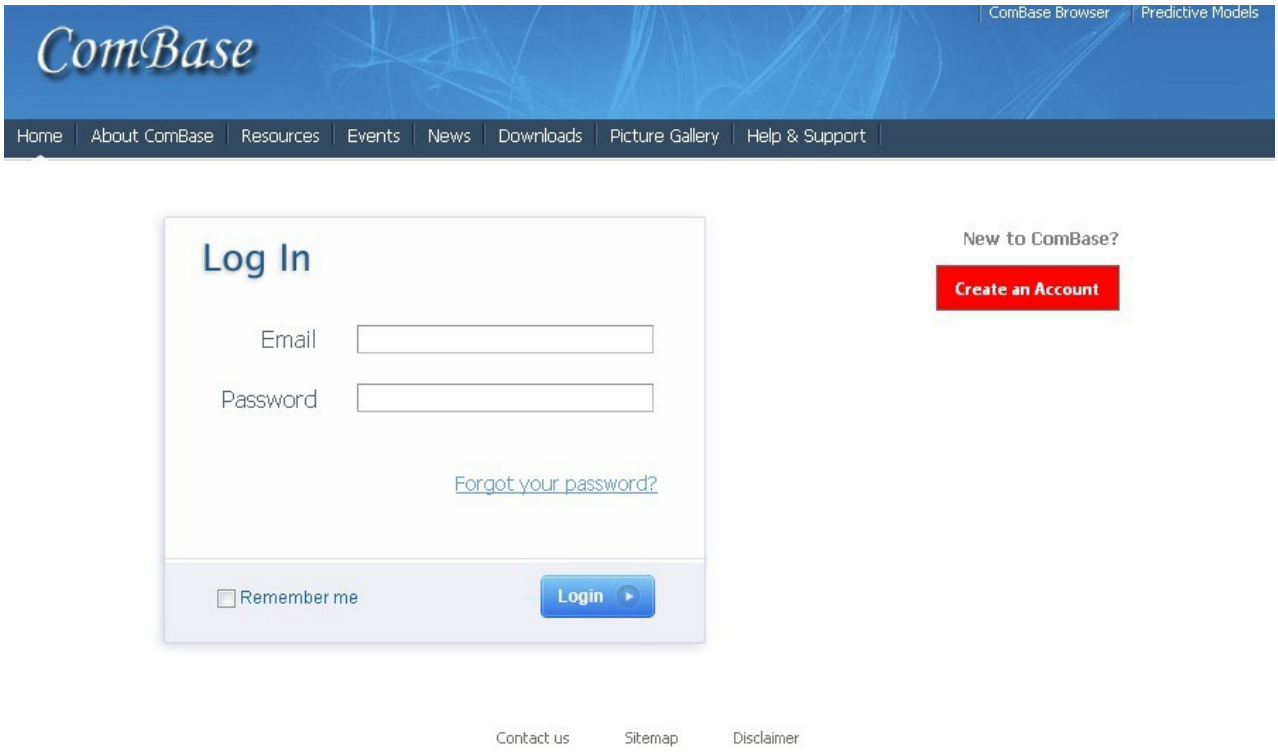
Figure 1



2.2 Login

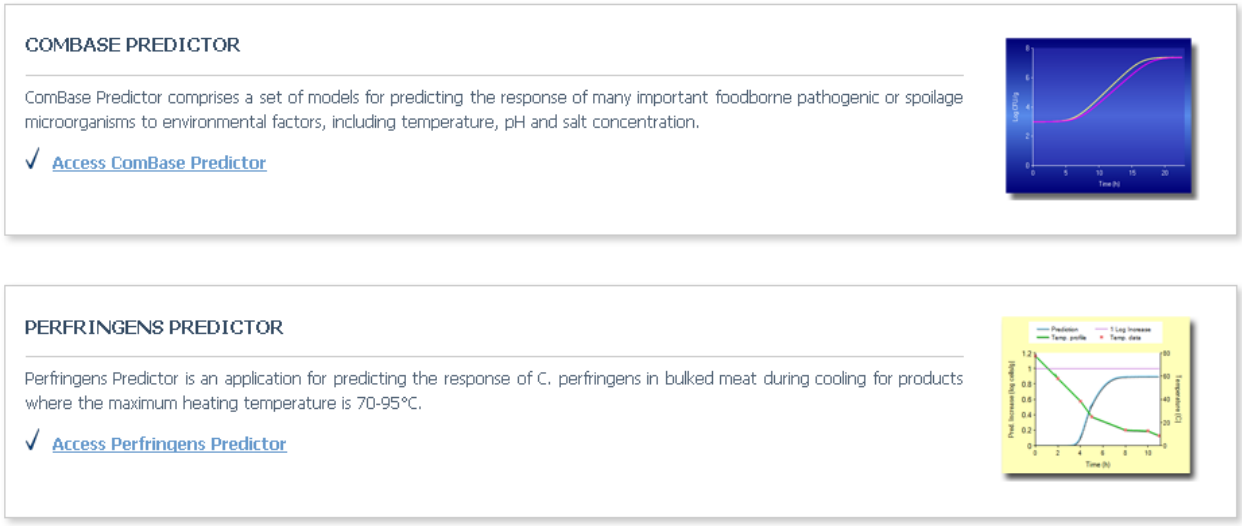
Once your account has been created, you can login by typing your email address and password as shown in Figure 2

Figure 2



Login in brings up the default **ComBase Predictive Tools** page (Figure 3).

Figure 3



Select ‘ComBase Predictor’ to access the *ComBase Predictor* page (Figure 4).

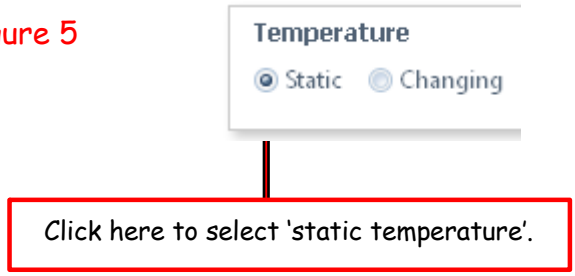
Figure 4

2.3 Using *ComBase Predictor*

2.3.1 Generating a single prediction at static temperature

The first step in producing a prediction is to select ‘static temperature’ in the temperature input field.

Figure 5

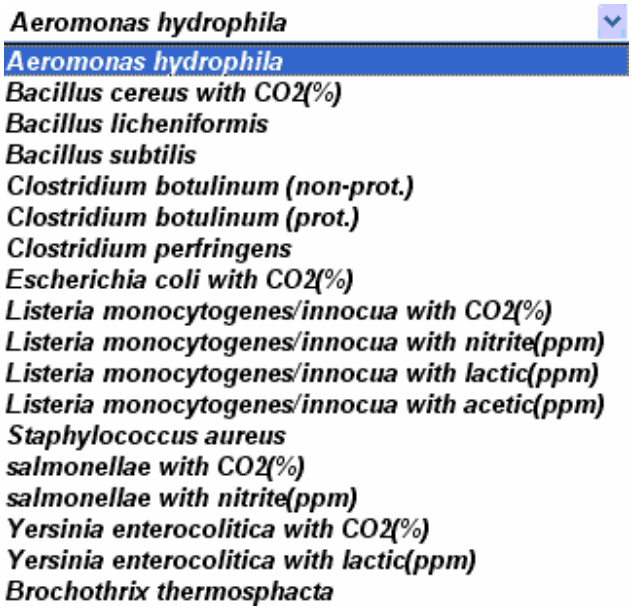


Select the model category: select 'growth model' for prediction of bacterial growth, 'thermal death model' for prediction of thermal inactivation, or ‘non thermal inactivation’.

Figure 6

Select the required model: The next step is to select a model. A drop-down menu of models included in *ComBase Predictor* can be viewed in the 'select a model' listbox. Click on the arrow to view the full list. The model required for the organism of interest can then be highlighted and selected from the menu.

Figure 7

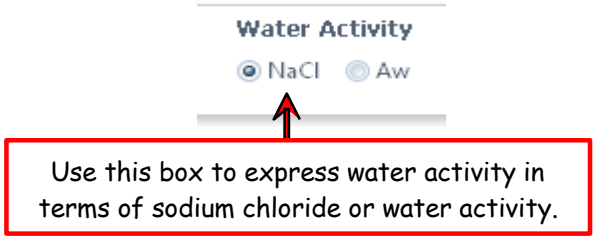


Many of the models encompass three environmental factors (temperature, pH and water activity). Some models also have an additional fourth environmental factor e.g. CO_2 or lactic acid. One basic principle of empirical modelling is that one should not extrapolate: predictions should not be made outside the region of observations. The limits of the selected model for each environmental factor appear below the input fields.

Input environmental factor values: Default values for the environmental factors (temperature, pH, water activity, etc) automatically appear in each of the input fields. Water activity can be expressed in terms of sodium chloride (%NaCl) or water activity (Figure 8). Water activity can be calculated from NaCl concentration by toggling between NaCl and Aw buttons (it is assumed that the salt is dissolved in water). The %NaCl values are transformed into water activity values by the formula:

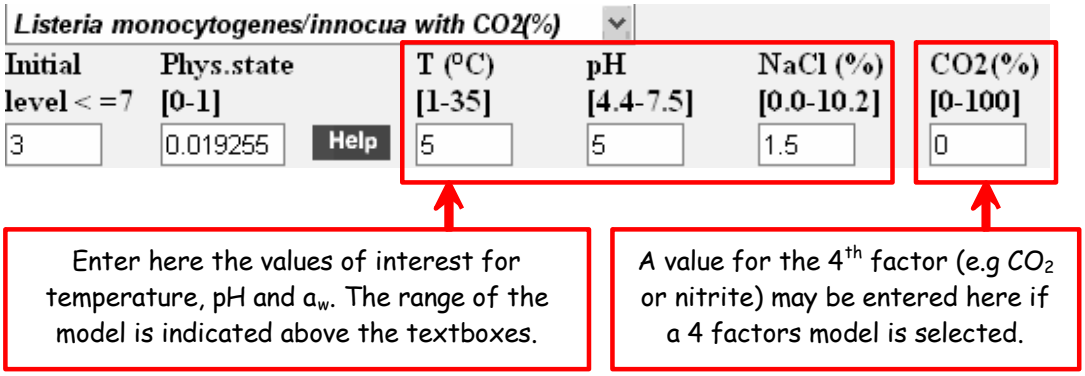
Aw=1-%NaCl*(5.2471+0.12206*%NaCl)/1000 (Resnik and Chirife, 1988)

Figure 8



Default values should be replaced by values of interest to you. The range within which input values must fall is indicated beside the input field for each of the factors. If values outside the range are selected, an error message will be generated.

Figure 9

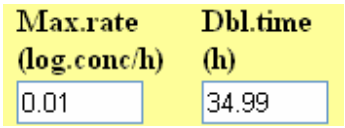


When a four-factor model has been selected, values are also required for the 'Factor 4' field. If preferred, the fields 'Initial level' (initial cell count expressed as $\log_{10}$ cfu/ml), 'Phys. state' (the physiological state of the cells expressed in terms of a value between 0 and 1) and 'Time (h)' (desired duration of observation) may be left empty; default values will then be inserted automatically by the program. The default value for the initial count is $\log_{10}$cell concentration = 3 (i.e. 10^3 cells/ml). For the physiological state, the default value is the value which was typical for the curves providing the base for the models. It is not possible to select 'initial level' values for thermal death models as results are provided as a relative decrease in log concentration.

To get a prediction: When all required values for the environmental factors have been satisfactorily selected, click 'Predict'.

The prediction output: The right-hand output panel now shows a graphical representation of the prediction. A statement of maximum growth rate and doubling time (or maximum death rate and D-value) are presented next to the input field.

Figure 10



Additionally, each of the time vs. cell concentration data points for the prediction are listed below the graph and may be selected and copied for use in other

applications (e.g. Excel). In the case of a death model, the output is not a predicted bacterial concentration but a relative log-decrease.

Figure 11

time(h)	conc.(Log10 cells/g)
0.00	3.00
0.00	3.00
23.22	3.00
46.44	3.01
69.66	3.02
92.88	3.04
116.10	3.07
139.32	3.11
162.54	3.17
185.76	3.24

2.3.2 Generating multiple predictions at static temperatures

Multiple predictions are generated in a similar manner to single predictions. Click the 'add a row' button up to three times to create multiple input sections.

Figure 12

A total of four simultaneous predictions can be made. Using this feature, it is possible to compare the responses of a number of different organisms to a single set of environmental conditions or for a single microorganism to varied environmental conditions. To return to a single prediction, click the 'remove last row' button the required number of times.

2.3.3 Generating a single prediction under fluctuating temperature

ComBase Predictor not only allows prediction to be made for static temperature conditions but also predictions under certain fluctuating (changing) temperature conditions. Note that this facility is available only for growth and thermal death models but not for non thermal inactivation model.

Predictions could be made for example from data representing the fluctuating time vs. temperature profile expected or measured for the following processes in sequence: the later stages of cooling, storage within the factory, storage during transportation, storage at retail premises, possible purchaser temperature abuse followed by domestic storage for the organisms *Listeria monocytogenes* and non-proteolytic *Clostridium botulinum* using the appropriate growth models. This 'fluctuating temperature' feature may also be useful to demonstrate the affect of a thermal process using logged data which falls within the relevant temperature range of the thermal death model.

Select 'changing temperature': Click the 'changing temperature' button. This will deselect 'static temperature' and generate the 'changing temperature' page.

Figure 13

Select the required model: Select the required model as described for static temperature predictions.

Input environmental factor values: Values for environmental factors for 'changing temperature' predictions should be entered as described for 'static temperature' predictions except for the values pertaining to temperature. To make predictions for 'changing temperature' situations, a time vs. temperature profile must be entered as the temperature parameter.

The time/ temperature should be inserted in the textbox on the 'changing temperature' page in the format illustrated and described below:

Format requirements:

1. Only the numeric characters (0-9), tabulation, space and return carriage are allowed in the textbox.
2. The records must be recorded in chronological order.
3. The first time-point must be '0'.
4. The last time point must be less than or equal to '5000' hours.

For each time temperature record of your profile, time is to be entered first (in hours) then temperature (in °C).

The 'point' symbol "." must be used as the decimal separator.

The profile can be typed directly into the ‘changing temperature' textbox as follows: value, space, value, return, (repeat for each line). Alternatively, the required data may be copied from another application (e.g. Excel spreadsheet, text file) and pasted directly into the text box.

Note also that all temperature values must be within the range for the models selected and that there must be a minimum of 4 and a maximum of 100 (time vs. temperature) records in the profile input.

To get a prediction: When all the required values and the temperature profile have been satisfactorily selected click ‘Predict’. If input data are incorrect an error message will appear. To continue with the prediction replace unacceptable data with data within the accepted range.

The prediction output: The right-hand output panel now shows time vs. cell concentration data for the given prediction (white) alongside the selected time vs. temperature profile (green). No statements of maximum growth rate or doubling time are provided as these fluctuate according to the time vs. temperature input. However, each of the time v. cell concentration data points for the prediction are listed below the graph and may be selected and copied for use in other applications.

2.3.4 Generating multiple predictions at fluctuating temperature

This process is performed in a similar manner to that already described. This facility can be used to compare organisms or key parameters for a single fluctuating temperature profile. It is not possible to make predictions when the temperature ranges of the models do not overlap.

Section 3: Modelling background

3.1 Introduction

Since the early 1980s, with the advent of powerful PCs, food safety research has increasing ly 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 was recognised by the UK 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* software, which predicted the growth and survival of various (mainly pathogenic) micro-organisms in food. These data now constitute the core of the database behind *ComBase Predictor*.

3.2 Data

Predictive modelling, for microbiological purposes, and therefore *ComBase Predictor,* is based on the recognition that, although food microbiology experiments may have been carried out for different purposes, such as challenge tests for new product development, or establishing the properties of an emerging pathogen, the results can always be put into the broad framework of "*Microbial response* to a specific *environment* around the cells".

Mathematically, this is a mapping between two spaces. Both can be characterised by one or more (possibly dynamic, i.e. time-dependent) factors depending on how detailed the description is intended to be.

Predictive models for *ComBase Predictor* are based only on output from laboratory experiments observed in culture media under well-controlled laboratory conditions. Data were not all considered with equal weight, since the aim was not to model the average outcome of those measurements, but to produce safe (conservative) predictions. Additionally, many data were obvious outliers, identified by mathematical / statistical means. However, these data were retained in the database (though not used in the creation of the models), since they can be useful to estimate the variability of microbial responses when experiments are repeated. These are the main reasons for observed growth curves being generally below predicted curves.

The raw data are stored in a Microsoft Access database, in a structure that was developed at IFR.

3.3 Mathematical modelling

The need to predict bacterial kinetics in food generated a discipline that is called Predictive Microbiology in the scientific literature. It focuses on the mathematical description of the microbial responses to the environment in food. A comprehensive review of the subject can be found in Ross *et al*. (1999), which also serves as a basis for this summary.

Predictive microbiology is based on the hypothesis that growth is an intrinsic characteristic of the organism and will occur reproducibly in the same environment. Variation of cell concentration is described by a mathematical

(growth or survival) curve and this is called a **primary modelSecondary models** describe how the parameters of primary models depend on environmental factors such as temperature, pH and water activity. These are described by mathematical functions and, by interpolation, the cell concentration against time can be predicted for any combination of conditions.

3.3.1 Primary models

3.3.1.1 A primary model for growth and thermal inactivation

Unlike the superseded *Food MicroModel*, which predominantly used the Gompertz sigmoid function for its primary growth model, *ComBase Predictor* uses the model of Baranyi and Roberts (1994). The main differences between the two approaches are explained below.
A sigmoid primary model is commonly described by four parameters:

(1) y_0 the natural logarithm of the initial cell concentration.
This is an initial value that is specific to the history of the organism and not related to the current growth environment.

(2) λ the time in lag phase (or shoulder).

The length of the lag phase will depend on the intrinsic characteristics of the bacteria, the current environmental conditions and the history (initial physiological state) of the cells. Cells that have come from a different environment or are damaged (for example after heat treatment or freezing) may require more time to synthesise macromolecules and repair damage before they can divide than undamaged cells from a similar environment.

(3) μ_{max} the maximum specific growth rate (or death rate).

This represents the steepest slope of the curve *cell concentration (natural logarithm) v. time*. It is considered to be an intrinsic characteristic of the organism dependant on the current environment but is not affected by the cell history. Note that the specific growth rate means the increase in natural logarithm per unit time, whilst the curves are shown with a logarithm to the base 10 of the cell concentration. Thus the real specific growth rate is, in fact, about 2.3-times higher than the growth rate seen on the plot.
The doubling time (required for the population to double in the exponential phase) is calculated as:

doubling time= ln(2)/specific growth rate.

The D-value (the time required for a 1-log reduction of the bacterial population in the death phase) is obtained as:

D-value= -ln(10)/specific death rate.

Note that all time values are in hours in the software.

(4) y_{max} the natural logarithm of the maximum cell concentration (or y_{min}, the logarithm of the concentration of the resistant population in the case of a death curve)

The maximum concentration is less extensively studied in predictive microbiology than the other growth parameters because food safety risk and spoilage generally appears at much lower concentrations; therefore, it is modelled only by an average value. In the case of thermal death models, y_{min} is not modelled in *ComBase Predictor* but considered to be y_{min}= -∞.

Using the above terminology:

1. The Gompertz function overestimates the maximum specific growth rate by 10-15%.

2. The maximum specific growth rate was the main model parameter for *Food MicroModel* and is the main model parameter for *ComBase Predictor*. However, in *ComBase Predictor*, the other one is not the lag but rather the "initial physiological state", α_0 (see Baranyi and Roberts, 1994). It 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 same role as the inoculum size: an initial parameter, quantifying the history of the cells (this parameter can be set up by the user).
From that, the lag is a consequence, as the following formula shows:

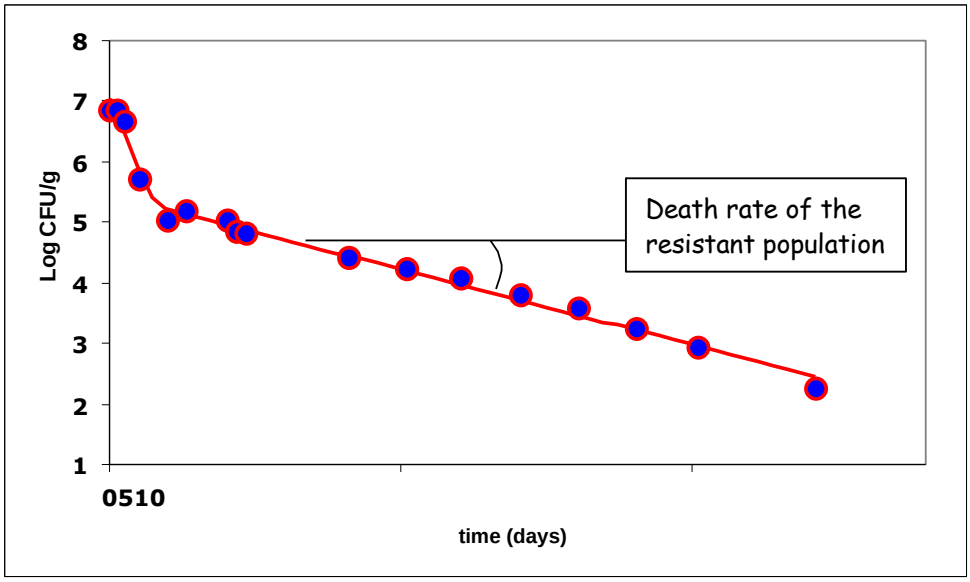
$$\lambda = -\ln(\alpha_0)/\mu_{max}$$

Because the user is rarely able to provide its value, a typical value is used as the default. Namely, if the chosen growth curves are fitted with the primary model, then the α_0 values obtained are modelled as function of the environmental factors (temperature, pH, water activity, possibly a fourth factor). This "secondary" model is simply a constant, taking the geometrical average of the $h_0 = -\ln(\alpha_0)$ values.

3.3.1.2 Primary models for non-thermal inactivation

The model of Baranyi and Roberts (1994) has also been used to describe inactivation curves with sigmoid shapes (or without shoulder or tail). The model of Baranyi and Roberts is inappropriate to describe some inactivation curves which do not exhibit this sigmoid shape. For example, for some curves, the death phase has a concave shape, due to the presence of a resistant population. Concave curves or concave curves followed by tailing were modelled with respectively biphasic (Figure 14) or triphasic models. In order not to underestimate microbiological risks, only the death rate of the resistant population was considered in the secondary modelling.

Figure 14



3.3.2 Secondary models

When creating the secondary models, the logarithms of the specific growth rates (or death rates) were described as a function of the (possibly rescaled) environmental factors by a standard quadratic multivariate polynomial. The %NaCl values were first transformed into water activity values by the formula:

a_w=1-NaCl*(5.2471+0.12206*NaCl)/1000

(Resnik and Chirife, 1988). For the growth and the non-thermal death models, the water activity values were rescaled as in Gibson *et al*. (1994) to obtain: b_w =sqrt(1-a_w).

For the models of *ComBase Predictor*, a standard second order polynomial modelled the effect of temperature, pH, and a_w (or b_w) values on the logarithm of the growth or death rate. This model was fitted for the rates produced by the primary models for each organism. They represent the **basic models**, which produce the "worst case" predictions, since further "fourth factors", such as CO_2 or nitrite content, only decrease these growth rates. (Note that other subsidiary conditions are also recorded in the database, but not taken into account for the predictions).
Currently only one "fourth factor" can be taken into account in any particular model. If the value of the fourth factor is 0, then the prediction produced by the basic three-factor model is obtained. This feature is currently lacking in any other predictive microbiology programme.

3.3.3 Model performance

Although modelling lag time would be preferable, to date, validated predictive models have been developed on growth rates. This is because the lag phase is technically difficult to study, so quantitative data is lacking, and lag is more

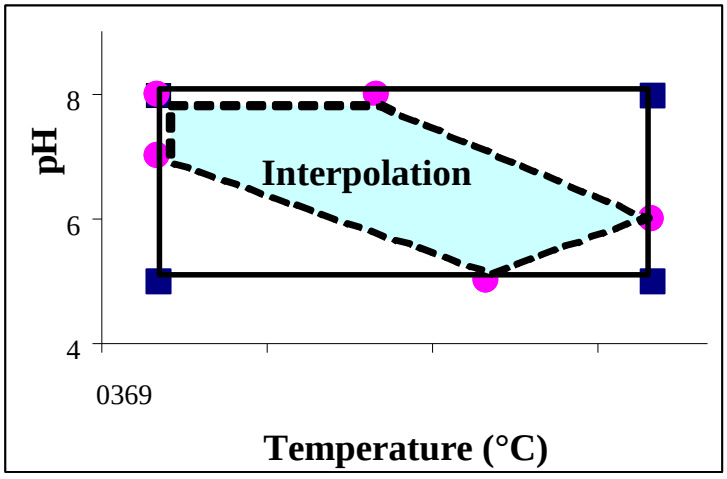
difficult to model as it depends not only on the current conditions but also on the history (or initial physiological state) of the cells.

To demonstrate the goodness of fit of the models, observed rates were plotted against predicted, for each model, in log-scale (see Appendix). The deviation of the points from the 45° equality line demonstrates the accuracy of the models. As a rule of thumb, the current performance of three-factor (core) predictive models is about 30-35 %, which means that the average error of prediction for the exponential growth / death rates is about this fraction of the respective predicted value.

One basic principle of empirical modelling is that one should not extrapolate, i.e. predictions should not be made outside the region of observations. However, in multidimensional environmental space, it can be quite difficult to determine what the interpolation region is. Figure 15 shows an example.

Suppose that growth rates were measured at those combinations of temperature and pH, which are denoted by the circles. Then, the squares are, in fact, extrapolated points, though their temperature and pH values, individually, are inside the interpolation intervals for each factor. Currently, *ComBase Predictor* considers a combination of environmental factors, and extrapolates only if any of the factors is outside the respective interval where observations were made. As can be seen in Figure 15, this is not always satisfactory. For example if observations were made at high temperature & low pH, and also at low temperature & high pH, then growth will be predicted at low temperature & low pH, where there is no evidence that it would occur.

Figure 15



The limits of the growth and thermal death models for each environmental factor for each organism are given in Table 4 and 5:

Table 4. The limits of each environmental factor in each growth model. The primary variables are temperature, pH and water activity (Aw); the fourth factor (ef4) can be either CO_2, nitrite, or organic acids, but not a combination of more of them.

Model Temp	$Temp_{min}$	$Temp_{max}$	pH_{min}	pH_{max}	Aw_{min}	$ef4_{max}$
Aeromonas hydrophila	2	37	4.6	7.5		0.974	0
Bacillus cereus with CO2(%)	5	34	4.9	7.4		0.94	60
Bacillus licheniformis	13	34	4	7.6		0.907	0
Bacillus subtilis	10	34	4.3	7.8		0.933	0
Clostridium botulinum (non-prot.)	4	30	5.1	7.5		0.974	0
Clostridium botulinum (prot.)	14	40	4.7	7.2		0.954	0
Clostridium perfringens	15	52	5	8	0.971	0	
Escherichia coli with CO2(%)	10	42	4.5	7.5		0.961	100
Listeria monocytogenes/innocua with CO2(%)	1	40	4.3	7.5		0.924	100
Listeria monocytogenes/innocua with nitrite(ppm)	1	40	4.4	7.5		0.924	200
Listeria monocytogenes/innocua with lactic(ppm)	1	40	4.4	7.5		0.924	20000
Listeria monocytogenes/innocua with acetic(ppm)	1	40	4.4	7.5		0.924	10000
Staphylococcus aureus	7.5	30	4.3	7.1		0.907	0
salmonellae with CO2(%)	7	40	3.9	7.4		0.973	100
salmonellae with nitrite(ppm)	7	40	3.9	7.4		0.973	200
Shigella flexneri with nitrite (ppm)	15	37	5.5	7.5		0.971	1000
Yersinia enterocolitica with CO2(%)	-1	37	4.4	7.2		0.957	80
Yersinia enterocolitica with lactic(ppm)	-1	37	4.4	7.2		0.957	10000
Brochothrix thermosphacta	0	30	5.5	7	0.95	0	
Pseudomonas spp	0	20	5	7.4		0.961	0

Notes: For each organism and model the upper limit of $A_w = 1$.
The fourth environmental factor is denoted by ef4, for which the minimum is always 0.

Table 5. The limits of each environmental factor in each thermal inactivation model. The primary variables are temperature, pH and water activity (Aw).

Model Temp	$_{min}$	$Temp_{max}$	pH_{min}	pH $_{max}$	Aw $_{min}$	Aw $_{max}$
Bacillus cereus	90	100	4.5	7	0.954	0.986
Clostridium botulinum (non-prot.)	80	95	4.1	7.3	0.971	1
Escherichia coli	54.5	64.5	4.2	8	0.947	1
Listeria monocytogenes/innocua	60	68	4.2	7	0.943	1
Salmonellae	54.5	65	4	7.1	0.997	1
Yersinia enterocolitica	52	60	4.2	7	0.961	1
Brochothrix thermosphacta	40	55	5	7	0.989	1

The limits of the non thermal inactivation models for each environmental factor for each organism are given in Table 6:

Table 6. The limits of each environmental factor in each non-thermal survival model. The primary variables are temperature, pH and water activity (Aw); the fourth factor (ef4) can be either CO_2, nitrite, or organic acids, but not a combination of them.

Model Temp	$_{min}$	$Temp_{max}$	pH_{min}	pH $_{max}$	Aw $_{min}$
Listeria monocytogenes/innocua	0	20	3.5	7	0.793
Salmonellae	0	40	4.3	7.5	0.781

Note: For each organism and model the upper limit of A_w = 1.

When the conditions of temperature, pH and water activity are also included in a growth model, an error message appears and the user is invited to continue with “Growth model”.

REFERENCES

Baranyi, J. and Roberts, T. A. (1994) A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology* 23, 277-294.

Baranyi J., Ross T., Roberts T.A. and McMeekin T. (1996). The effects of overparameterisation on the performance of empirical models used in Predictive Microbiology. Food Microbiol. 13. 83-91

Baranyi J. , Tamplin M. (2002). ComBase: A Common Database on Microbial Responses to Food Environments. J. Food Prot. (In press).

Gibson A. M., Baranyi J., Pitt I., Eyles M. J. and Roberts T. A. (1994). Predicting fungal growth: the effect of water activity on four species of *Aspergillus*. *International Journal of Food Microbiology* 23, 419-431.

Pin C. and Baranyi J. (1998). Predictive models as means to quantify the interactions of spoilage organisms. Int.J. Food Microbiol. 41. 59-72.

Resnik, S. L. and Chirife, J. (1988). Proposed theoretical aw values at various temperatures for selected solutions to be used as reference sources in the range of microbial growth. *Journal of Food Protection* 51, 419-423.

Ross, T.A. and McMeekin, T.A. (1994). Predictive Microbiology. Int.J. Food Microbiol. 23. 241-264.

Ross T., Baranyi J. and McMeekin T. A. (1999). Predictive Microbiology and Food Safety. In: Encyclopaedia of Food Microbiology, eds: Robinson, R., Batt, C. and Patel, P. Academic Press.

Appendix

A. Data source

A.1. Growth models

Aeromonas hydrophila (3 factors)

Core model (temperature, pH, aw): 125 rates from the Food Micro Model data set, 31 rates from Palumbo et al. (1991), 2 rates from Golden et al. (1989)

References

Palumbo S. A., Willimas A.C., Buchanan R.L. and Phillips J.G. 1991: Model for the aerobic growth of *Aeromonas hydrophila* K144. J. Food Prot. 54, 429-435.

Golden D.A., Eyles M. J., and Beuchat L. R. 1989: Influence of modified atmosphere storage on the growth of uninjured and heat injured *Aeromonas hydrophila*. Appl. Environ. Microbiol. 55(11), 3012-3015.

Bacillus cereus with CO2(%)

Core model (temperature, pH, aw): 86 rates from the Food Micro Model data set, 76 rates from the Institute of Food Research (Norwich, UK), 33 rates from London Metropolitain University.

CO_2 data: 58 rates from the Food Micro Model data set.

Bacillus licheniformis (3 factors)

Core model (temperature, pH, aw): 58 rates from the Food Micro Model data set.

Bacillus subtilis (3 factors)

Core model (temperature, pH, aw): 68 rates from the Food Micro Model data set.

Clostridium botulinum (non-prot.) (3 factors)

Core model (temperature, pH, aw):52 rates from the Food Micro Model data set.

Clostridium botulinum (prot.) (3 factors)

Core model (temperature, pH, aw): 77 rates from the Food Micro Model data set.

Clostridium perfringens (3 factors)

Core model (temperature, pH, aw): 51 rates from the Food Micro Model data set.

Escherichia coli with CO2(%)

Core model (temperature, pH, aw): 80 rates from the Food Micro Model data set, 72 data from Buchanan et al. (1992) and 17 data from Glass et al. (1992).

CO_2 data: 78 rates from the Food Micro Model data set.

References

Buchanan R.L., Klawitter, L. A. 1992. The effect of incubation temperature, initial pH and sodium chloride on the growth kinetics of *Escherichia coli* O157:H7. Food Microbiol. 9: 185 - 196

Glass K. A., Loeffelholz J. M., Ford J. P., and. Doyle M. P. 1992. Fate of Escherichia coli O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Appl. Environ. Microbiol. 58(8), 2513 - 2516

Listeria monocytogenes/innocua with CO2(%)

Core model (temperature, pH, aw): 251 rates from the Food Micro Model data set, 206 data from Buchanan and Phillips (1990), 25 data from Duh et al. (1993), 43 data from Le Marc (2001).

CO_2 data: 75 rates from the Food Micro Model data set, 9 data from the Institute of Food Research, Norwich.

References

Buchanan R. L. and Phillips J. G. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Protect. 53, 370-376.

Duh Y. H. and Schaffner D. W. 1993. Modelling the effect of temperature on the growth rate and lag time of *Listeria innocua* and *Listeria monocytogenes*. J. Food Protect. 56, 205-210.

Le Marc Y. Développement d'un modèle modulaire décrivant l'effet des interactions entre les facteurs environnementaux sur les aptitudes de croissance de *Listeria*. Thèse de doctorat. Université de Bretagne Occidentale, France.

Listeria monocytogenes/innocua with nitrite(ppm)

Data (temperature, pH, aw): 251 rates from the Food Micro Model data set, 206 data from Buchanan and Phillips (1990), 25 data from Duh et al. (1993), 43 data from Le Marc (2001).

Nitrite data: 75 rates from the Food Micro Model data set, 118 data from Buchanan and Phillips (1990).

References

Buchanan R. L. and Phillips J. G. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Protect. 53, 370-376.

Duh Y. H. and Schaffner D. W. 1993. Modelling the effect of temperature on the growth rate and lag time of *Listeria innocua* and *Listeria monocytogenes*. J. Food Protect. 56, 205-210.

Le Marc Y. Développement d'un modèle modulaire décrivant l'effet des interactions entre les facteurs environnementaux sur les aptitudes de croissance de *Listeria*. Thèse de doctorat. Université de Bretagne Occidentale, France.

Listeria monocytogenes/innocua with lactic(ppm)

Data (temperature, pH, aw): 251 rates from the Food Micro Model data set, 206 data from Buchanan and Phillips (1990), 25 data from Duh et al. (1993), 43 data from Le Marc (2001).

Lactic acid data: 129 rates from the Food Micro Model data set.

References

Buchanan R. L. and Phillips J. G. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Protect. 53, 370-376.

Duh Y.H. and Schaffner D. W. 1993. Modelling the effect of temperature on the growth rate and lag time of *Listeria innocua* and *Listeria monocytogenes*. J. Food Protect. 56, 205-210.

Le Marc Y. Développement d'un modèle modulaire décrivant l'effet des interactions entre les facteurs environnementaux sur les aptitudes de croissance de *Listeria*. Thèse de doctorat. Université de Bretagne Occidentale, France.

Listeria monocytogenes/innocua with acetic(ppm)

Data (temperature, pH, aw): 251 rates from the Food Micro Model data set, 206 data from Buchanan and Phillips (1990), 25 data from Duh et al. (1993), 43 data from Le Marc (2001).

Acetic acid data: 52 rates from the Food Micro Model data set.

References

Buchanan R. L. and Phillips J. G. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Protect. 53, 370-376.

Duh Y. H. and Schaffner D. W. 1993. Modelling the effect of temperature on the growth rate and lag time of *Listeria innocua* and *Listeria monocytogenes*. J. Food Protect. 56, 205-210.

Le Marc Y. Développement d'un modèle modulaire décrivant l'effet des interactions entre les facteurs environnementaux sur les aptitudes de croissance de *Listeria*. Thèse de doctorat. Université de Bretagne Occidentale, France.

Staphylococcus aureus (3 factors)

Core model (temperature, pH, aw): 93 rates from the Food Micro Model data set.

salmonellae with CO2(%)

Core model (temperature, pH, aw): 146 rates from the Food Micro Model data set, 9 data from Bovill et al. (2000) and 104 data from Oscar (1999).

CO_2 data: 75 rates from the Food Micro Model data set.

References

Bovill R., Bew J., Cook N., D'Agostino M., Wilkinson N. and Baranyi J. 2000. Predictions of growth for *Listeria monocytogenes* and Salmonella during fluctuating temperature. Int. J. Food Microbiol. 59, 157-165.

Oscar T.P.1999. Response surface models for effects of temperature, pH, and previous growth pH on growth kinetics of *Salmonella typhimurium* in brain heart infusion broth. J. Food Protect. 62,106-111.

salmonellae with nitrite(ppm)

Core model (temperature, pH, aw): 146 rates from the Food Micro Model data set, 9 data from Bovill et al. (2000) and 104 data from Oscar (1999).

Nitrite data: 28 rates from the Food Micro Model data set.

References

Bovill R., Bew J., Cook N., D'Agostino M., Wilkinson N. and Baranyi J. 2000: Predictions of growth for *Listeria monocytogenes* and Salmonella during fluctuating temperature. Int. J. Food Microbiol. 59, 157-165.

Oscar T.P.1999: Response surface models for effects of temperature, pH, and previous growth pH on growth kinetics of *Salmonella typhimurium* in brain heart infusion broth. J. Food Protect. 62, 106-111.

Shigella flexneri with nitrite(ppm)

Core model (temperature, pH, aw): 193 data from Zaika et al. (1992).

Nitrite data: 125 rates from Zaika et al. (1992).

Reference

Zaika L. L., Phillips J. G. and Buchanan R.L. 1992. Model for aerobic growth of *Shigella flexneri* under various conditions of temperature, pH, sodium chloride and sodium nitrite concentrations. J. Food Protect. 55, 509-513.

Yersinia enterocolitica with CO2(%)

Core model (temperature, pH, aw): 282 rates from the Food Micro Model data set, 12 rates from Alber et al. (1992) and 23 data from Badhuri et al. (1995).

CO_2 data: 31 rates from the Food Micro Model data set.

References

Alber S. A. and Schaffner D. W. 1992. Evaluation of data transformations used with the square root and Schoolfield models for predicting bacterial growth rate. Appl. Environ. Microbiol. 58, 3337-3342.

Bhaduri S., Buchanan R. L. and Phillips J. G.1995. Expanded response surface model for predicting the effects of temperatures, pH, sodium chloride contents and sodium nitrite concentrations on the growth rate of *Yersinia enterocolitica*. J. Appl. Bacteriol. 79,163-170.

Yersinia enterocolitica with lactic(ppm)

Core model (temperature, pH, aw): 282 rates from the Food Micro Model data set, 12 rates from Alber and Schaffner (1992) and 23 data from Badhuri et al. (1995).

Lactic acid data: 77 rates from the Food Micro Model data set.

References

Alber S. A and Schaffner D. W. 1992: Evaluation of data transformations used with the square root and Schoolfield models for predicting bacterial growth rate. Appl. Environ. Microbiol. 58, 3337-3342.

Bhaduri, S., Buchanan, R. L. and Philips J. G.1995. Expanded response surface model for predicting the effects of temperatures, pH, sodium chloride contents and sodium nitrite concentrations on the growth rate of *Yersinia enterocolitica*. J. Appl. Bacteriol. 79,163-170.

Pseudomonas spp. (3 factors)

Data (temperature, pH, aw): 12 rates from Pin et al. (1998), 70 rates from Campden and Chorlwywood Research Association, Chipping Campden and 62 rates from London Metropolitan University.

Reference

Pin C. and Baranyi J. 1998. Predictive models as means to quantify the interactions of spoilage organisms. Int.J. Food Microbiol. 41. 59-72.

Brochothrix thermosphacta (3 factors)

Core model (temperature, pH, aw): 44 rates from the Food Micro Model data set.

A.2. Thermal death models

Bacillus cereus (3 factors)

Core model (temperature, pH, aw): 41 death rates from the Food Micro Model data set.

Clostridium botulinum (non-prot.) (3 factors)

Core model (temperature, pH, aw): 35death rates from the Food Micro Model data set.

Escherichia coli (3 factors)

Core model (temperature, pH, aw): 51 rates from the Food Micro Model data set, 21 data from Unilever Research.

Listeria monocytogenes (3 factors)

Core model (temperature, pH, aw): 67 rates from the Food Micro Model data set, 13 data from Unilever Research; 136 data from Linton et al. (1995).

Reference

Linton, R.H., Carter W.H., Pierson M.D, Hackney C.R. and Eifert J.D. 1995. Use of a modified gompertz equation to predict the effects of temperature, pH, and NaCl on the inactivation of *Listeria monocytogenes* Scott A heated in infant formula. J. Food Protect. 59, 16-23

Salmonellae (3 factors)

Core model (temperature, pH, aw): 61 rates from the Food Micro Model data set, 8 data from ARS-Eastern Regional Research Center, Philadelphia, USA.

Yersinia enterocolitica (3 factors)

Core model (temperature, pH, aw): 86 death rates from the Food Micro Model data set.

Brochothrix thermosphacta (3 factors)

Core model (temperature, pH, aw): 53 death rates from Baranyi et al. (1996).

References

Baranyi J., Jones A., Walker C., Kaloti A. and Mackey B.M. 1996. A combined model for growth and thermal inactivation of *Brochothrix thermosphacta*. J. Appl. Env. Microbiol. 62. 1029-1035.

A.3. Non thermal inactivation models

Listeria monocytogenes (3 factors)

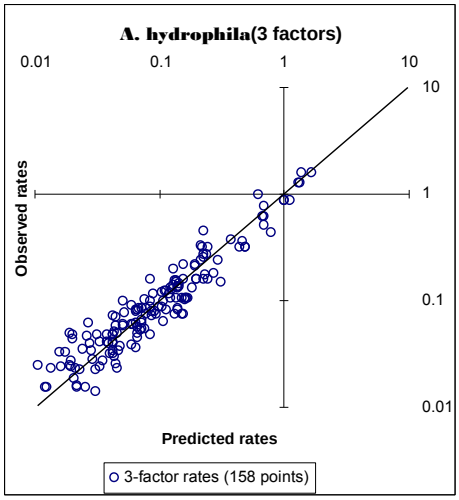
Data (temperature, pH, aw): 194 inactivation rates from the Food Micro Model data set.

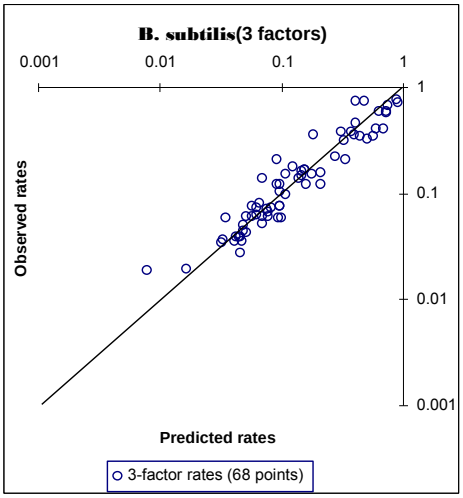
Salmonellae (3 factors)

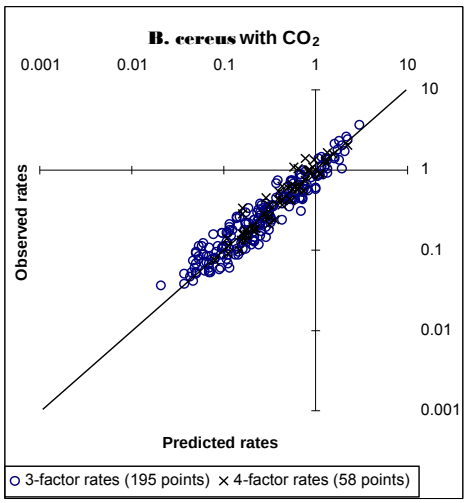
Data (temperature, pH, aw): 102 inactivation rates from the Food Micro Model data set.

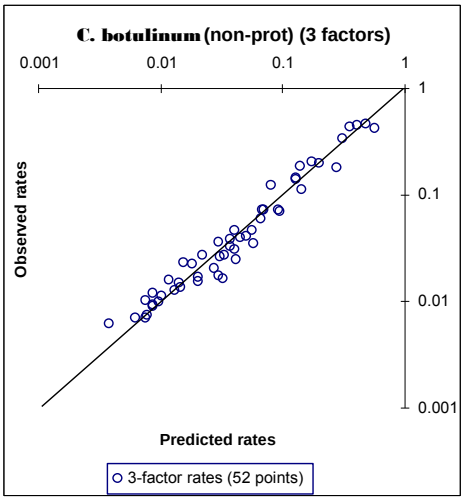
B. Demonstrating the goodness of fit for each model

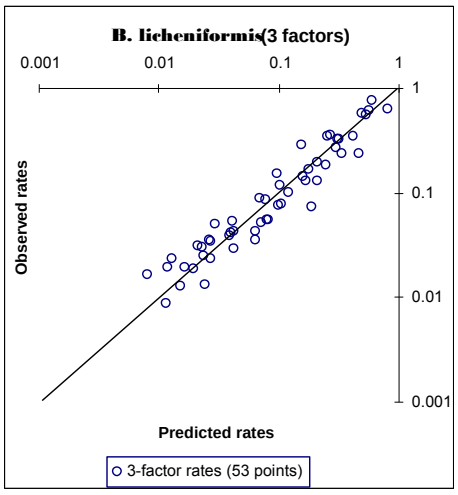
B.1. Growth models

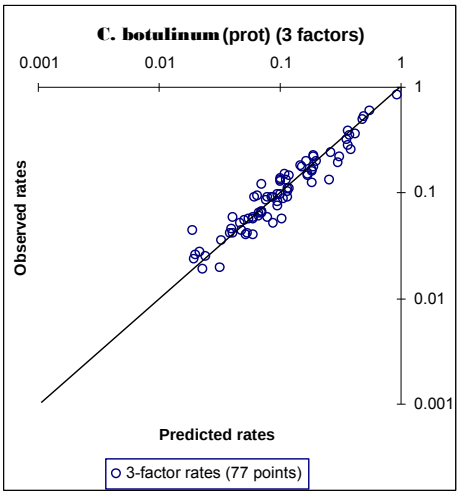


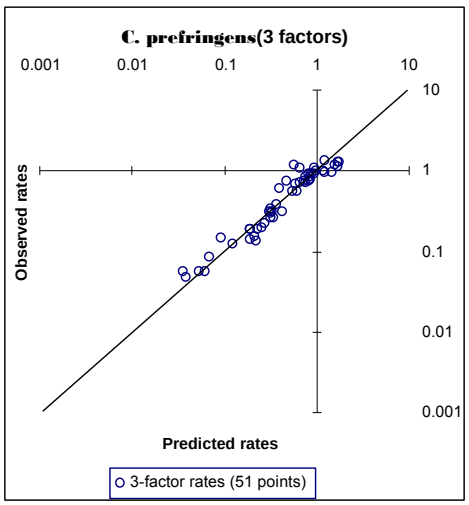


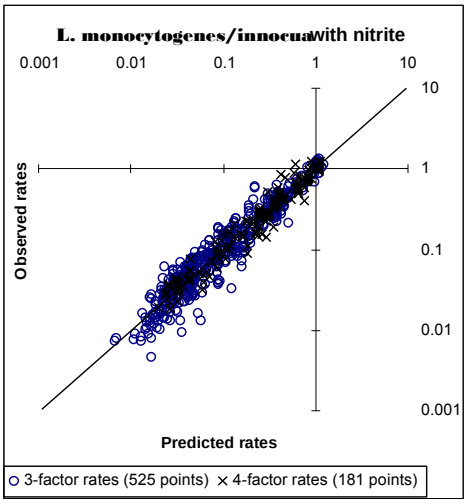


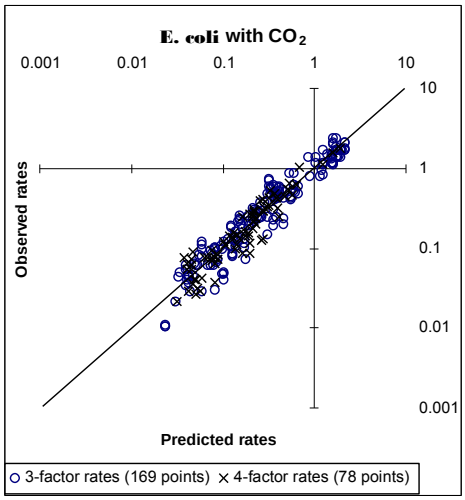


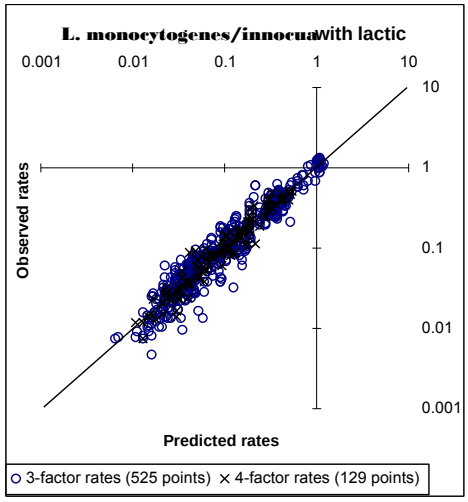


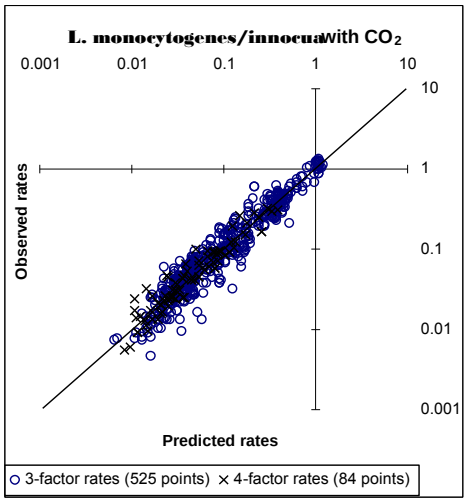


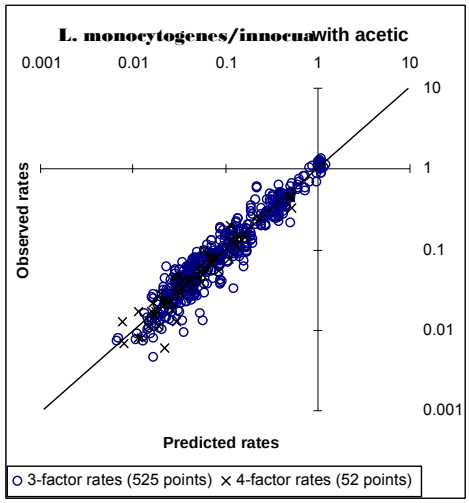

C. prefringens(3 factors)
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (51 points)

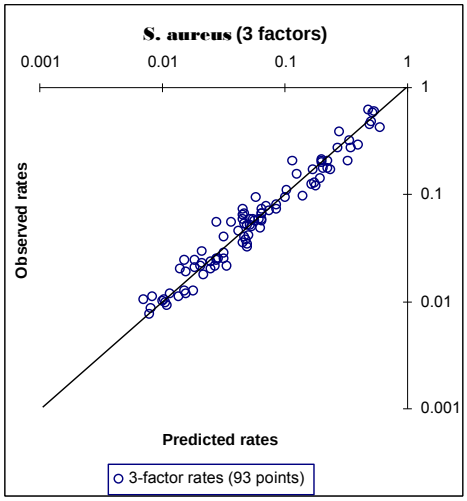

L. monocytogenes/innocua with nitrite
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (525 points) × 4-factor rates (181 points)

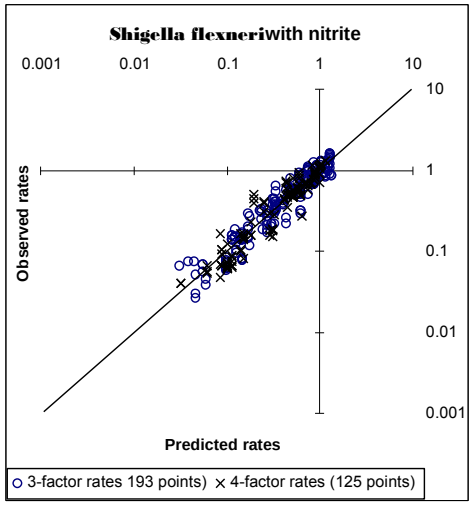

E. coli with CO2
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (169 points) × 4-factor rates (78 points)

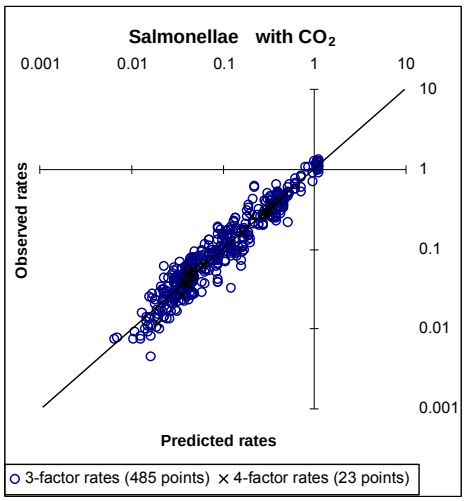

L. monocytogenes/innocua with lactic
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (525 points) × 4-factor rates (129 points)

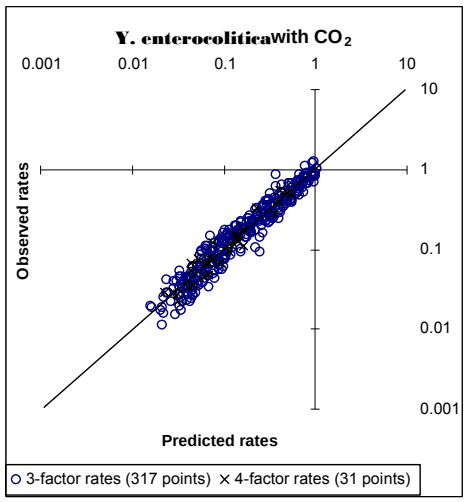

L. monocytogenes/innocua with CO2
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (525 points) × 4-factor rates (84 points)

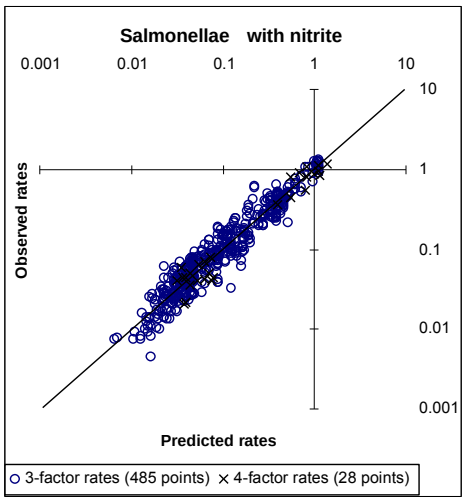

L. monocytogenes/innocua with acetic
0.001
0.01
0.1
1
10
Observed rates
Predicted rates
3-factor rates (525 points) × 4-factor rates (52 points)

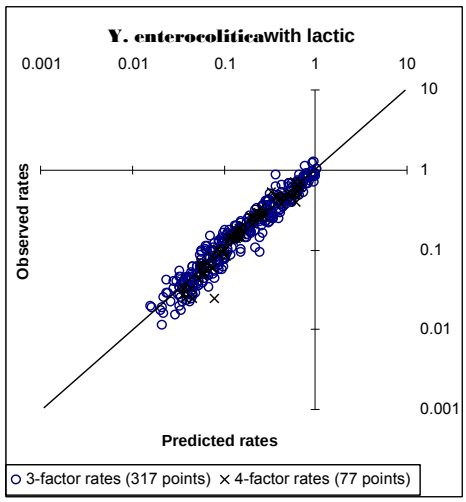

S. aureus (3 factors)
0.001
0.01
0.1
1
1
0.1
0.01
0.001
Observed rates
Predicted rates
3-factor rates (93 points)

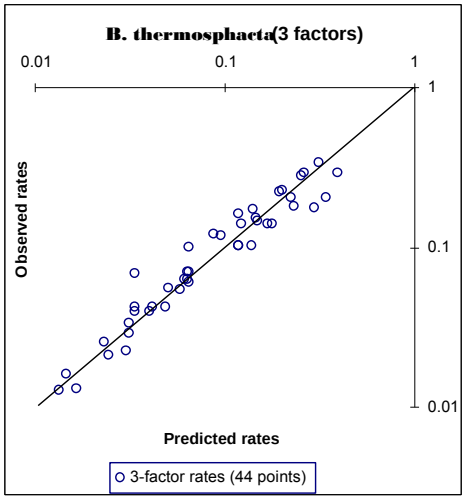

Shigella flexneriwith nitrite
0.001
0.01
0.1
1
10
10
1
0.1
0.01
0.001
Observed rates
Predicted rates
3-factor rates 193 points) × 4-factor rates (125 points)

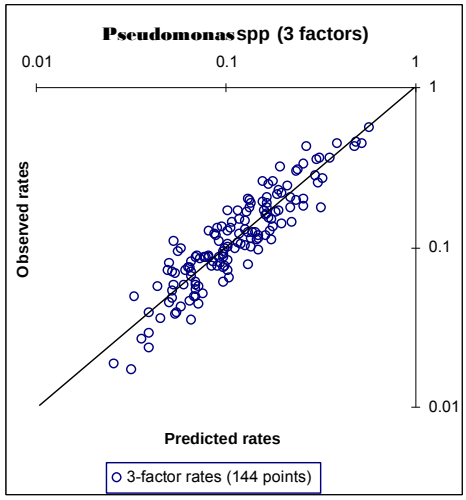

Salmonellae with CO2
0.001
0.01
0.1
1
10
10
1
0.1
0.01
0.001
Observed rates
Predicted rates
3-factor rates (485 points) × 4-factor rates (23 points)


Y. enterocoliticawith CO2
0.001
0.01
0.1
1
10
10
1
0.1
0.01
0.001
Observed rates
Predicted rates
3-factor rates (317 points) × 4-factor rates (31 points)


Salmonellae with nitrite
0.001
0.01
0.1
1
10
10
1
0.1
0.01
0.001
Observed rates
Predicted rates
3-factor rates (485 points) × 4-factor rates (28 points)


Y. enterocoliticawith lactic
0.001
0.01
0.1
1
10
10
1
0.1
0.01
0.001
Observed rates
Predicted rates
3-factor rates (317 points) × 4-factor rates (77 points)


B. thermosphacta (3 factors)
0.01
0.1
1
1
0.1
0.01
Observed rates
Predicted rates
3-factor rates (44 points)


Pseudomonas spp (3 factors)
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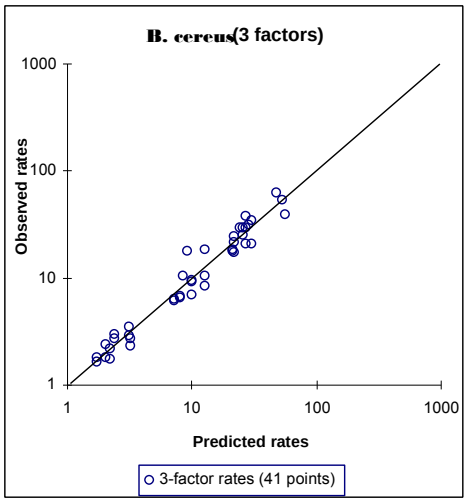
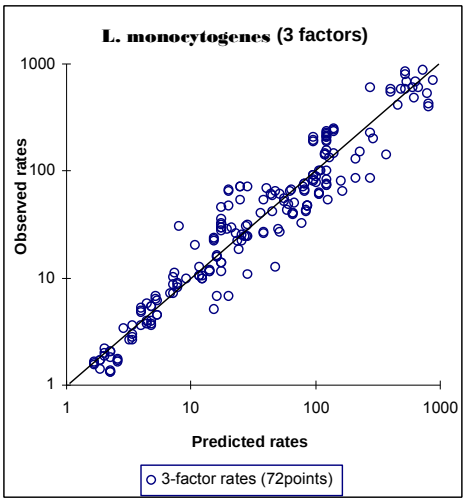
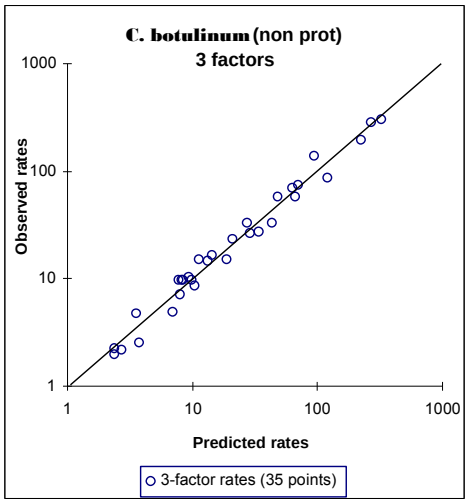
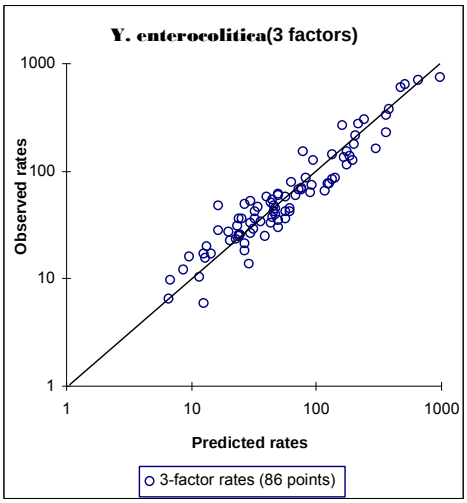
0.1
1
1
0.1
0.01
Observed rates
Predicted rates
3-factor rates (144 points)

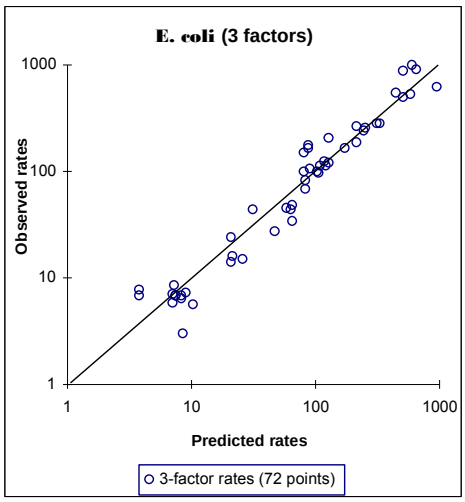
B.2. Thermal death models

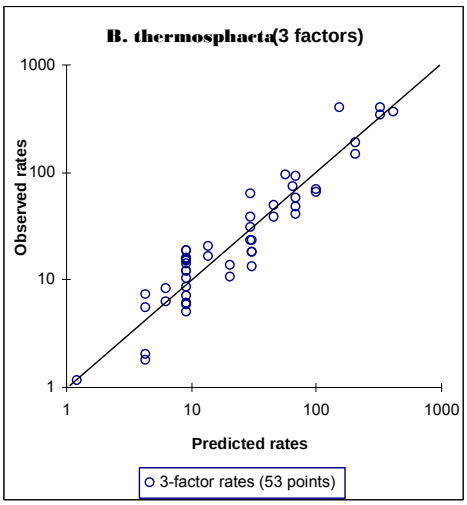












B.3.Non thermal inactivation models