Introduction to USDA Integrated Pathogen Modeling Program (IPMP) 2013

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DISCLAIMER AND ASSUMPTION OF RISK

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Please contact Dr. Lihan Huang (Lihan.Huang@ars.usda.gov) for technical questions.

SUGGESTED CITATION

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INTRODUCTION

What is IPMP 2013?

IPMP 2013 is a new generation predictive microbiology tool. It is designed to analyze experimental data commonly encountered in predictive microbiology and for the development of predictive models.

Why IPMP 2013?

Modern predictive microbiology has significantly evolved since the 1980's. While progress has been achieved in predictive microbiology research, there is, however, no comprehensive data analysis and model development tool. Many researchers use commercial general-purpose statistical analysis and mathematical tools, such as SAS®, Matlab®, Mathematica®, S-Plus®, or SPSS®, while others use open-source statistical analysis tools, such as R, for data analysis and model development. Unfortunately most of these general-purpose tools require program-specific programming. For someone lacking programming knowledge, it can be difficult to use these tools effectively. Additionally, commercial statistical packages and math tools are expensive. IPMP 2013 is developed by USDA-ARS to meet the needs of the predictive microbiology scientific community. Offered as a free tool, IPMP is a simple-to-use data analysis platform for developing predictive models. With IPMP 2013, anyone, with a basic knowledge of predictive microbiology, can use it to analyze kinetic data and develop predictive models for microorganisms.

What can IPMP 2013 do?

IPMP 2013 is a data analysis tool developed to analyze the kinetic data of microbial growth and inactivation frequently found in predictive microbiology. It is specifically designed to develop primary and secondary models, and contains user-friendly interfaces that allow the user to enter and analyze kinetic data by selecting certain mathematical models.

What is required to use IPMP 2013?

All the statistical analysis and model development are handled seamlessly behind the scenes. No programming knowledge is needed. The user only needs to enter the data and click a few buttons on the screen to complete any data analysis. The only requirement is that the user have a basic knowledge of predictive microbiology to allow for the selection of suitable models for data analysis.

What models are included in IPMP 2013?

IPMP 2013 was developed to analyze primary and secondary models. More complex models may be included in the future. The primary models include common growth and inactivation models. They can be used to analyze full growth curves (containing all three phases), incomplete growth curves, or inactivation/survival curves. The secondary models are used to evaluate the effect of temperature on growth rate.

STRUCTURE of IPMP 2013

Components

Once IPMP 2013 is initiated, an introduction screen (Figure 1) will appear. It shows the contact information for the product. Once the introduction screen disappears, IPMP 2013 will be loaded. IPMP 2013 consists of 4 independent floating windows (Figure 2). Each window can be independently dragged, expanded, shrunk, or closed.

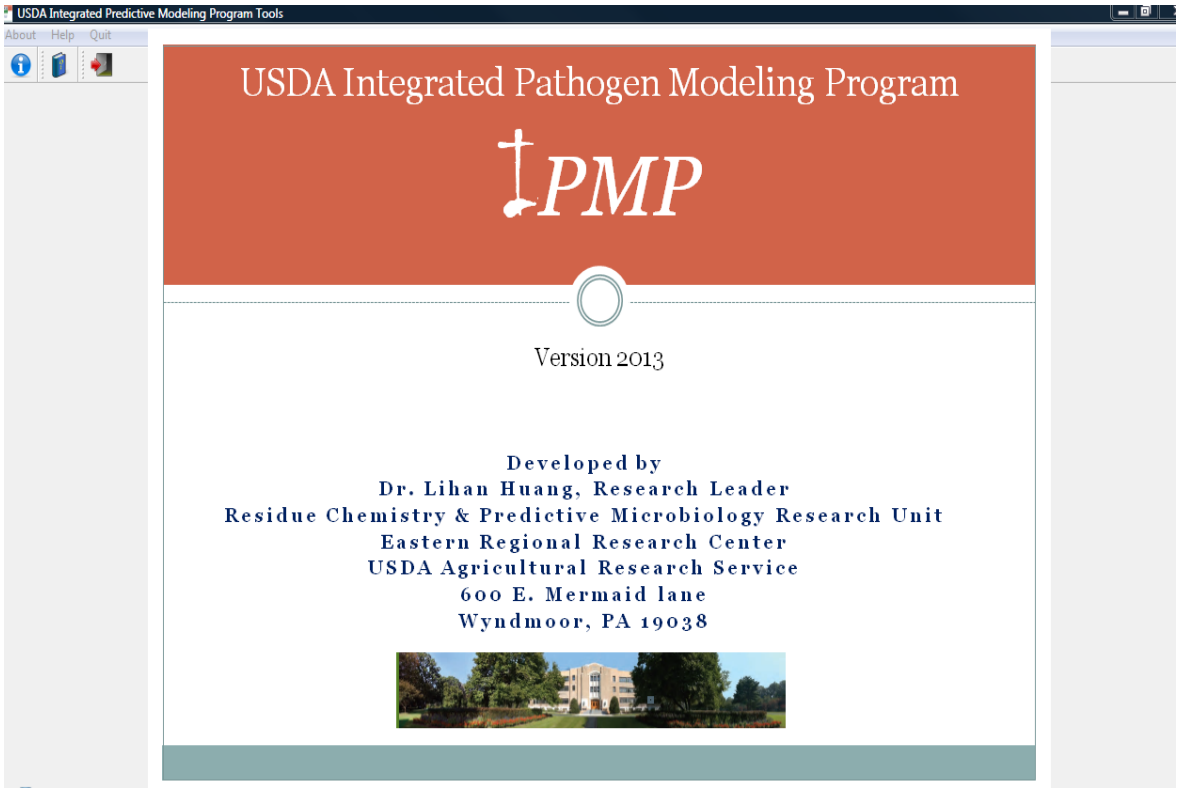

Figure 1. Introduction screen (about screen).

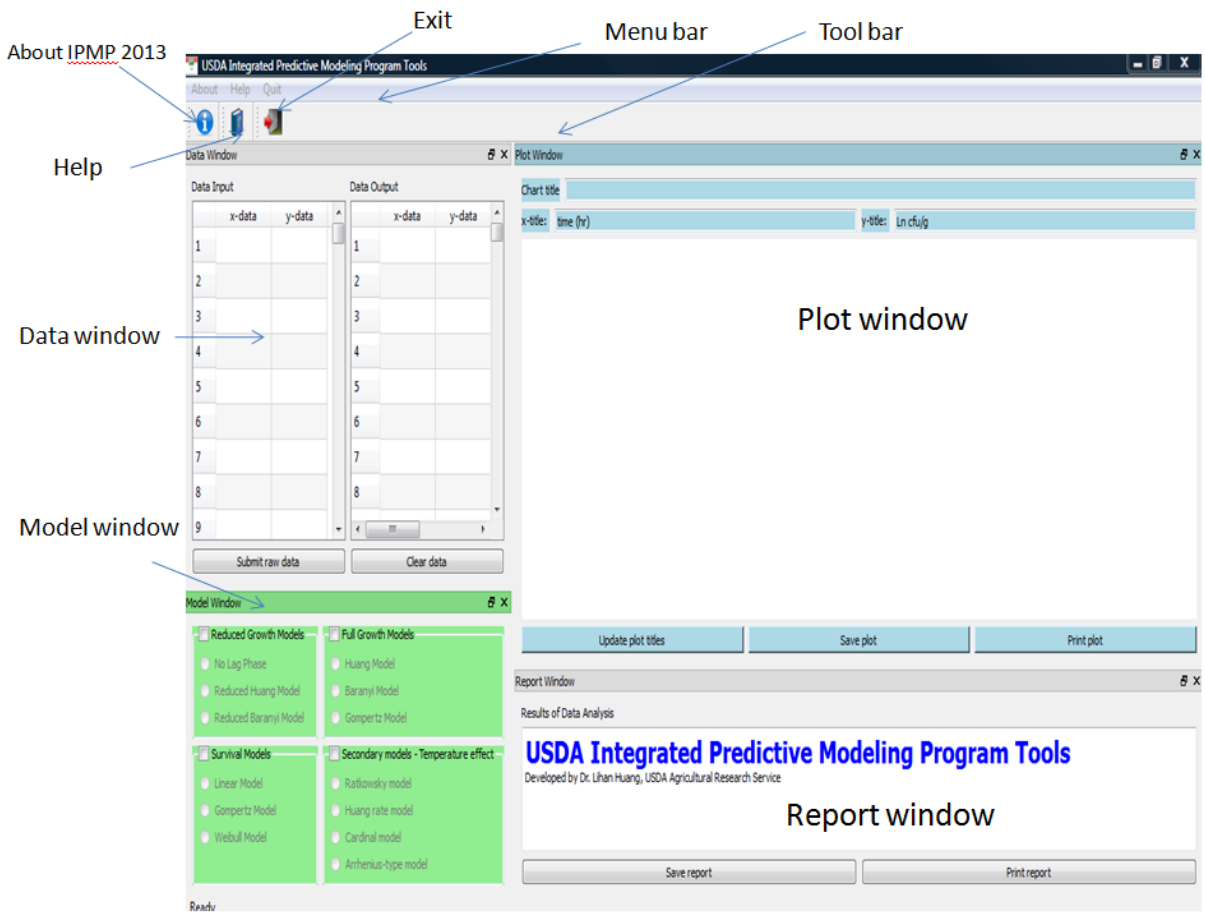

Figure 2. Main window and its components.

Window manipulation

To expand or shrink a window, place the cursor between two windows until appears. Drag to expand or shrink (Figure 3). Click 'X' in each window to close a window. Click the double squares next to 'X' to make a window float (Figure 4). A floating window can be dragged and repositioned anywhere in the main window. The remaining windows will automatically adjust as the floating window is repositioned.

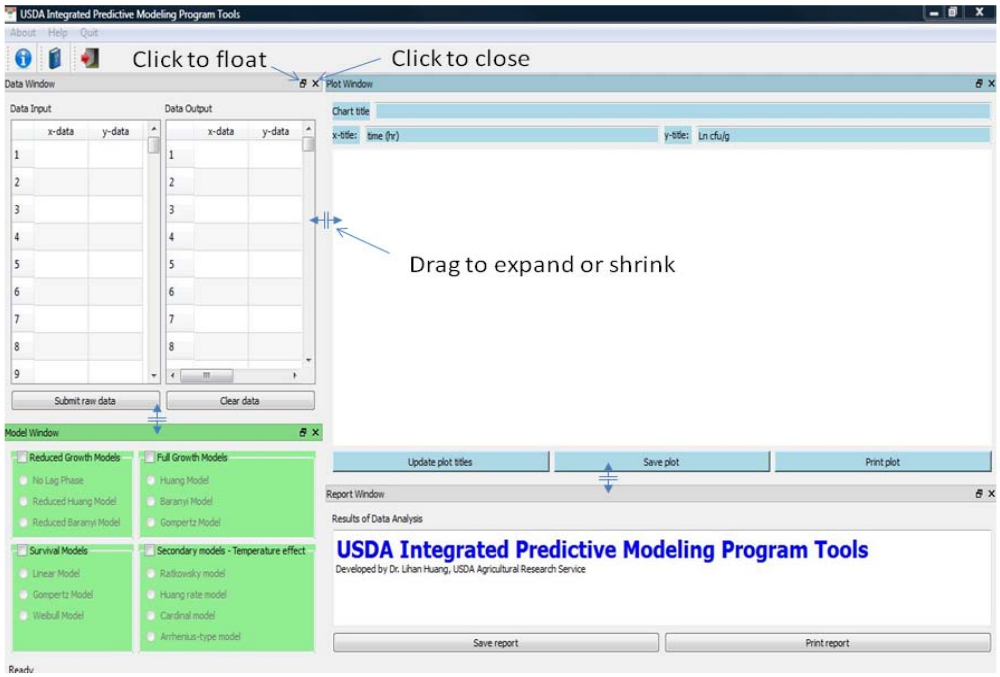

Figure 3. Expand, shrink, or make a window float.

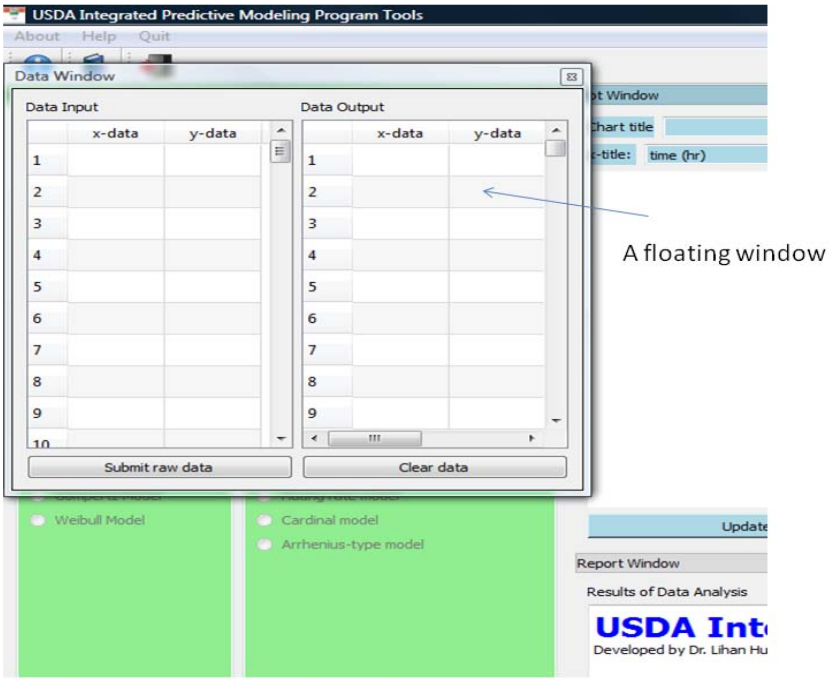

Figure 4. A floating window.

If any window is accidentally closed, it can be restored by right-clicking the tool bar area (Figure 5). A new menu will pop-up. Make selections in the pop-up menu. The closed window will be restored immediately.

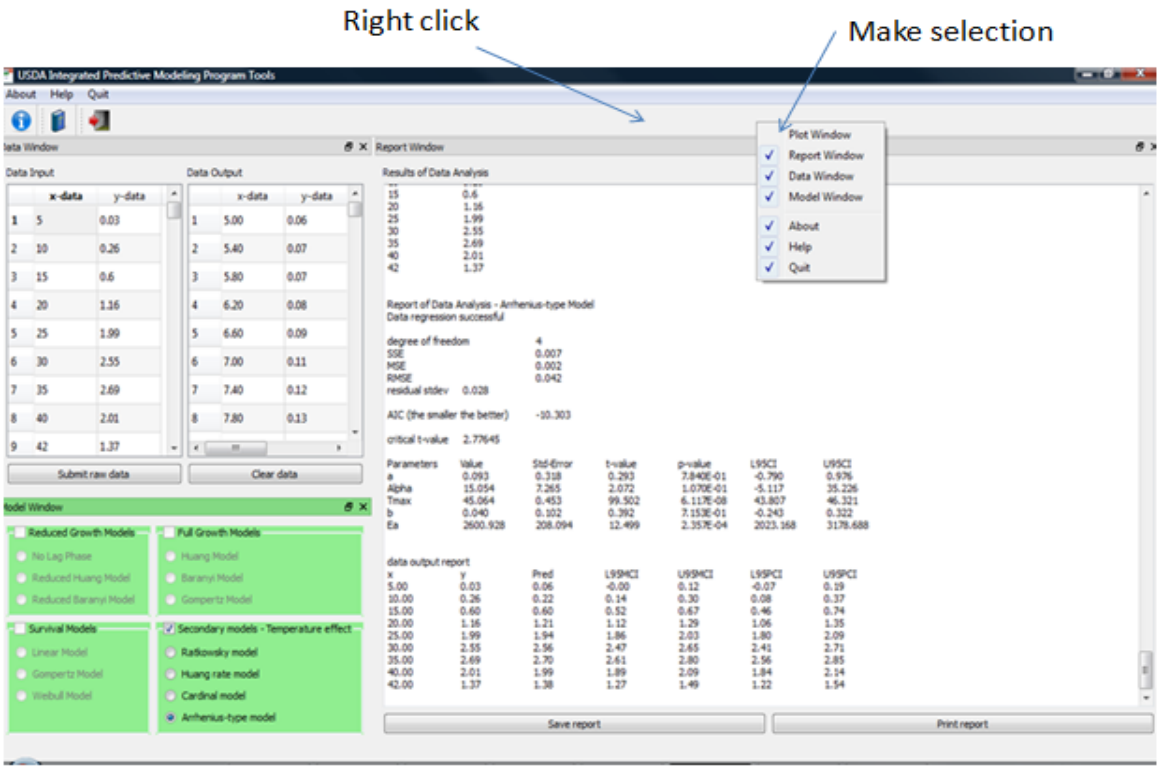

Figure 5. Restore closed windows.

DATA WINDOW

Components

The data window contains a spreadsheet-style input area and output area (Figure 6). The data input area contains two columns and 100 rows, and the output area contains two columns and 1000 rows. The raw data should be entered to the input area. Each area can be scrolled to examine the data.

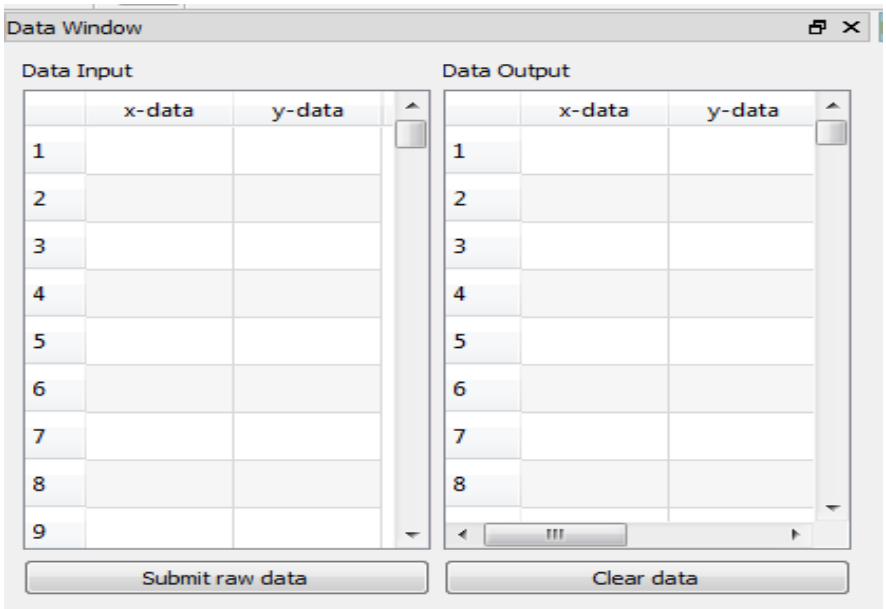

Figure 6. Data window.

Raw Data Entry

Raw data must be entered in the data input area. The raw data can be directly entered from the keyboard or copied/pasted from a text editor or a spreadsheet (Excel®, for example). The data can be growth or survival data. For growth models (except the Gompertz model and the two-phase/three-phase linear model), all analyses will be based on the **Natural Logarithms** of bacterial counts. If the data are bacterial populations, the x data can be time, and the y data can be log10 or the natural logarithms (Ln or ln) of bacterial counts (cfu/g, or cfu/ml). Once data entry is completed and submitted, a dialog will appear for data conversion. Only two columns of data can be entered into the data input area. To paste the data, right click the mouse in the raw data table, choose “paste” to paste the data. The data can be pasted by pressing ‘Ctrl-V” in the Windows Operating System. Once the data entry is complete, click “submit raw data”. The raw data will be automatically plotted in the Plot Window. **It is only necessary to click “submit raw data” ONCE**. The program contains a basic mechanism to check for missing data or non-numerical data. Only numeric values can be entered in the data input area. Do not enter the variable names. **No missing data are allowed. The number of x data must be equal to the number of y data.**

Raw data can be edited by right-clicking the mouse. The edit operations include “cut”, “copy”, “paste”, and “clear”. The data can be save to “cvs” format by clicking the “save” option.

If necessary, click “Clear data” to erase the data from the input area. A dialog will appear to confirm if the data are to be cleared. Once confirmed, the data will be cleared from the memory, and the Plot Window will be reset accordingly. Once data entry is complete, continue to Model Window for data analysis.

Clear Raw Data

Once data analysis is complete, it is necessary to clear the data before new data can be entered, which can be accomplished by clicking “Clear data”. Again, a dialog will appear to confirm if the data are to be cleared. Once confirmed, the data will be cleared from the memory, and the Plot Window will be reset accordingly.

MODEL WINDOW

Components and model selection

The Model Window consists of four groups of models (Figure 7). Each of them is mutually exclusive, i.e., only one group can be selected at each time. To select a group of models, click the square selection box next to the title of each group. Once a group is selected, the rest of the model groups will be disabled. You have to click the square box to unselect the selected group before you can select another group.

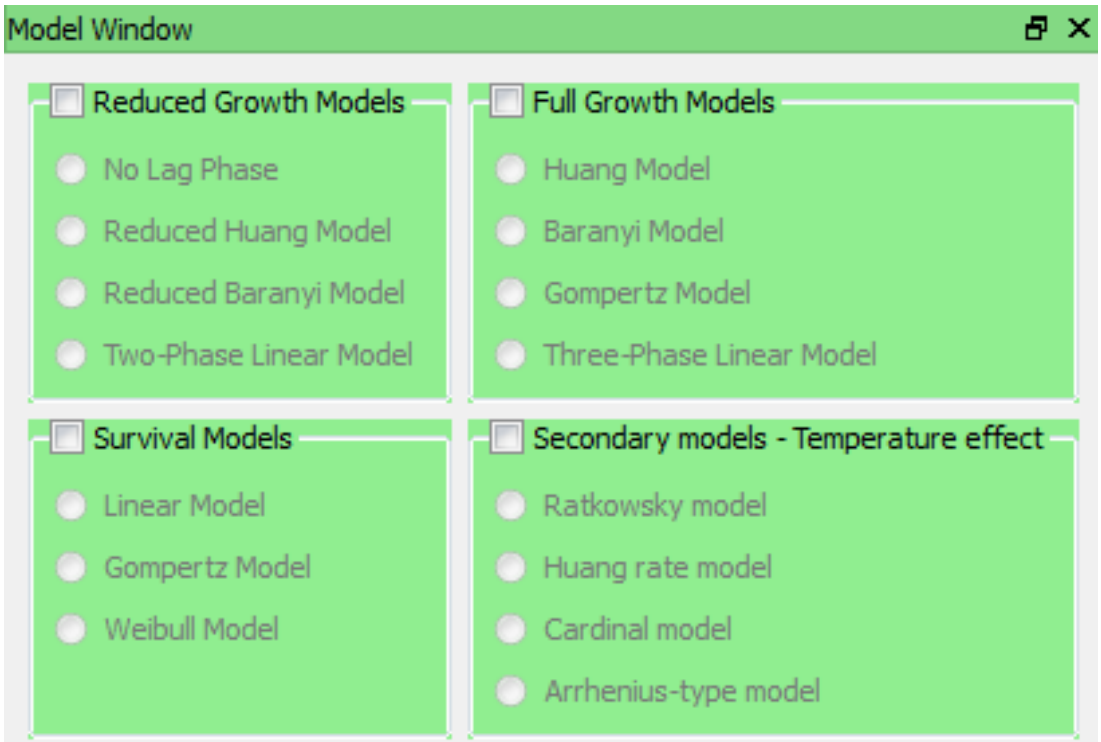

Figure 7. Model Window.

Adjustment of initial parameters

Once a group is selected, you can choose a model by clicking one of the radio buttons. Once a model is selected, a window will pop up (Figure 8), and a preliminary curve will be plotted. The pop up window contains the parameters for each model. Each parameter can be adjusted by adjusting the slider or the spin box. The number of parameters depends on the model. Once a parameter is adjusted, the preliminary curve will be automatically adjusted. Adjust the slider, spin box, or the text area to adjust the parameter until the preliminary curve is fine-tuned, when the preliminary curve closely matches the raw data (Figure 9). This exercise allows nonlinear regression to converge faster. Once the parameter(s) is fine-tuned, click the "Submit Model" button. The data will be submitted to the data analysis engine for processing. **For linear inactivation model, no pop up window will appear.** Once data analysis is complete, the model curve will be plotted.

The data plot can be saved or printed by clicking the “Save plot” or “Print plot” button. The title of the plot, x axis, and y-axis can be changed by entering text in the areas above the plot. Click ‘Update plot title” to make the changes.

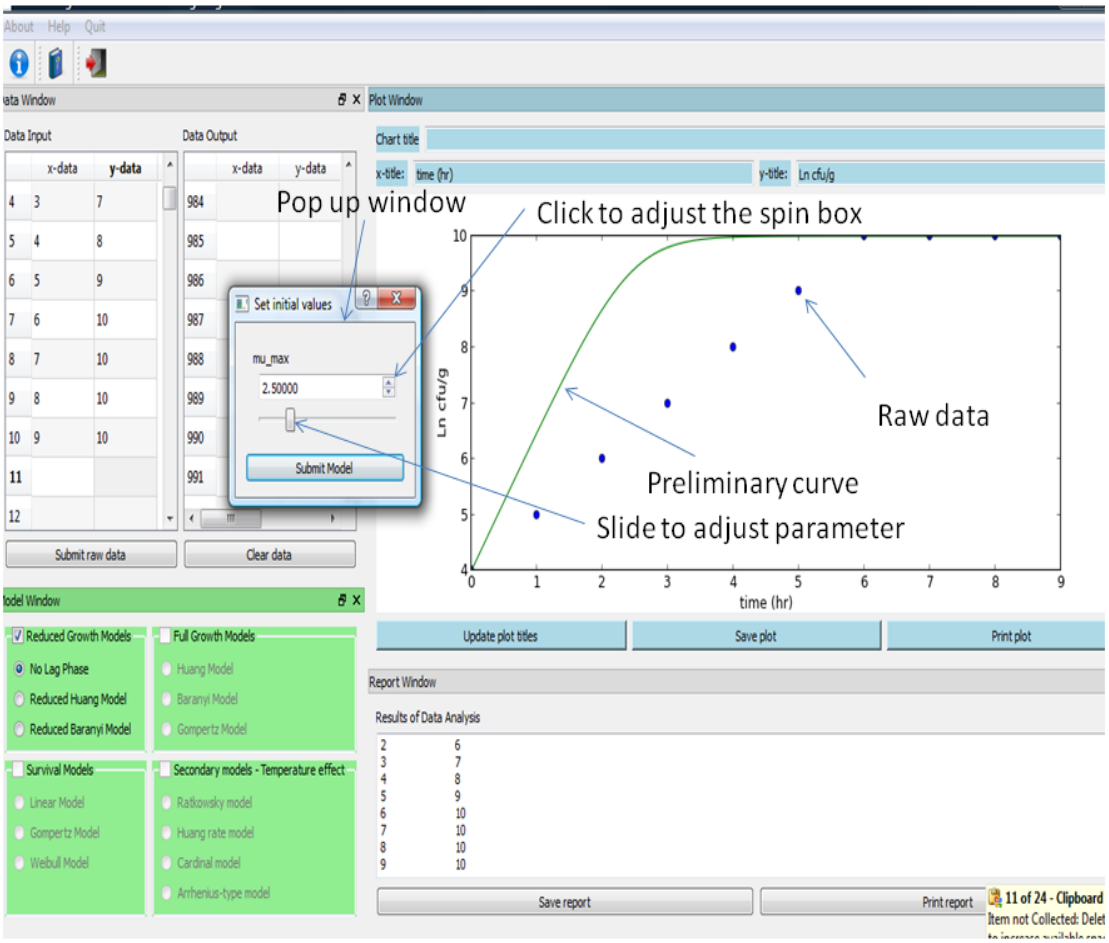

Figure 8. Parameter adjustment Pop up Window.

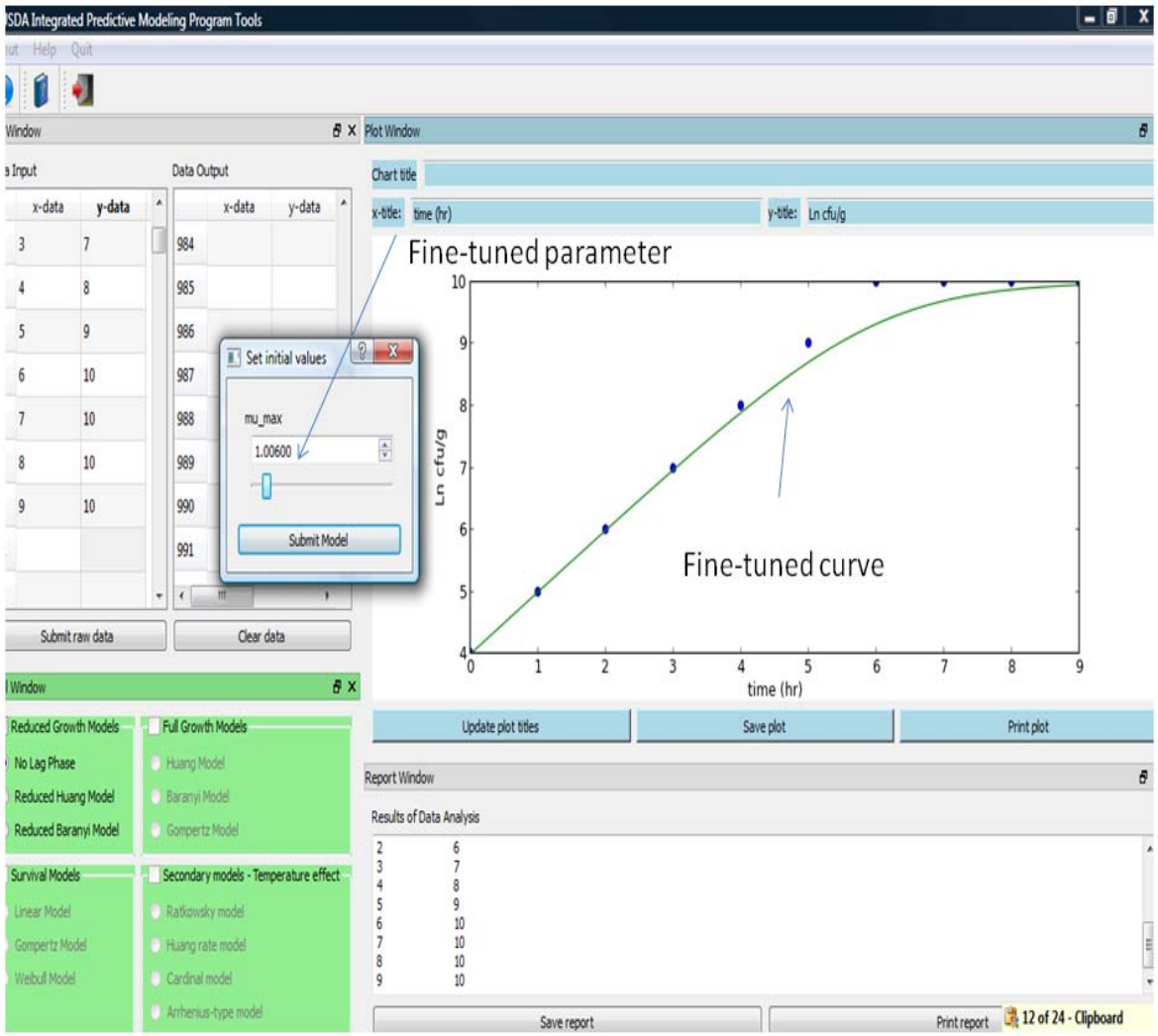

Figure 9. Fine-tuned parameter.

DATA REPORT WINDOW

Window components

The data report window is a text reporting area to display the results of analysis (Figure 10). Within this window, there is a button for saving the text report and another for printing. Once the data are submitted, they will be automatically sent to the report window, along with the time when the data are submitted (Figure 11). After the data analysis is complete, the results will also be sent to the report window (Figure 12). The report can be saved or printed by clicking the buttons below the text area.

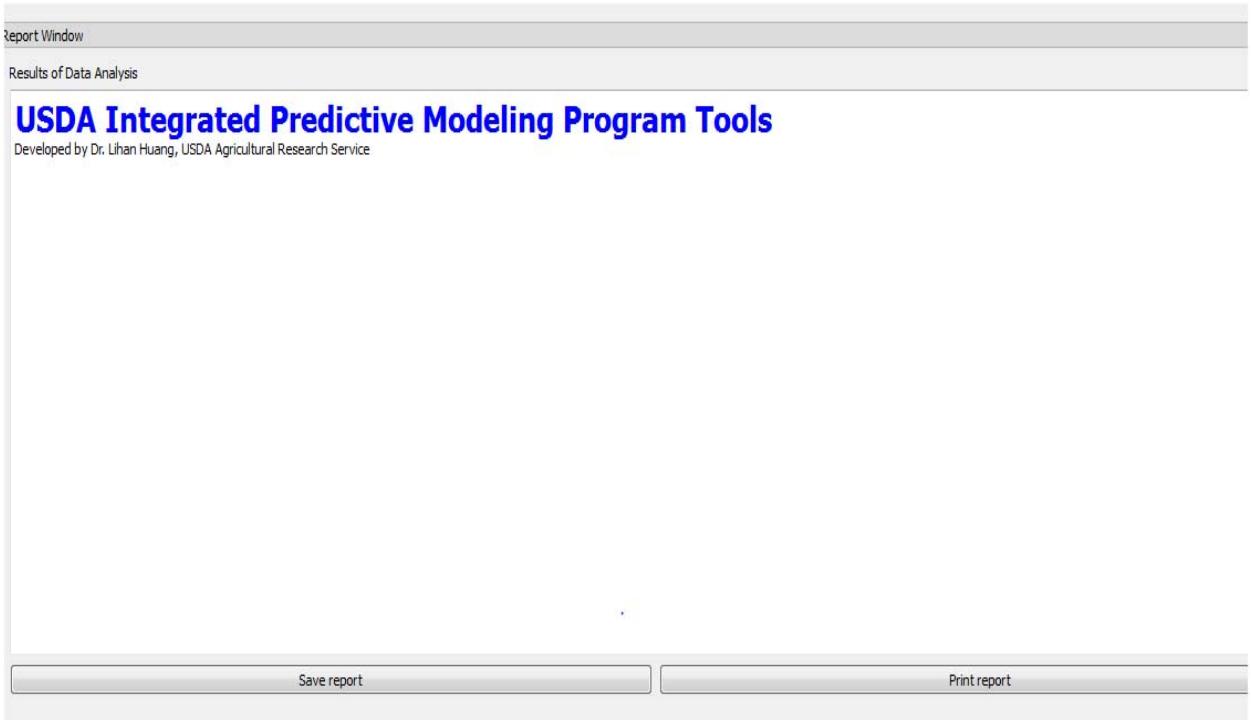

Figure 10. A blank data report window.

Results of Data Analysis

USDA Integrated Predictive Modeling Program Tools

Developed by Dr. Lihan Huang, USDA Agricultural Research Service

Current time: Tue Apr 09 11:08:14 2013

Raw data

x	y
0	3
1	4
2	5
3	6
4	7
5	8
6	9
7	10
8	10
9	10

Figure 11. Raw data in the report window.

Figure 12. Data analysis report.

Data report components

n: number of data points in a curve.

p: number of parameters in a model.

df: degree of freedom, n – p.

SSE: sum of squared errors, $\sum_{0}^{n-1}(y_i - \widehat{y_i})^2$.

MSE: mean of SEE, SSE/df.

RMSE: square root of MSE.

Residual standard deviation: standard deviation of errors.

AIC: Akaike information criterion, $n \times ln\left(\frac{SEE}{n}\right) + 2(p+1) + \frac{2(p+1)(p+2)}{df-2}$, df > 2 (Brul, van Gerwen, and Zwietering et al., 2007).

Parameters: parameters in an equation to be determined by linear or nonlinear regression.

L95CI and U95CI: lower and upper 95% confidence interval for the estimated parameters.

L95MCI and U95MCI: approximate lower and upper 95% confidence intervals for the expected value (mean) (SAS, 2013).

L95PCI and U95PCI: approximate lower and upper 95% confidence intervals for individual prediction (SAS, 2013).

MATHEMATICAL MODELS IN IPMP 2013

Group 1 – Reduced Growth models

1. No lag phase (Fang, Gurtler, and Huang, 2012; Fang, Liu, and Huang, 2013)
Equation:

$$\boldsymbol{Y(t) = Y_0 + Y_{max} - ln[e^{Y_0} + (e^{Y_{max}} - e^{Y_0})e^{-\mu_{max}t}]} \qquad \text{Eq. 1}$$

This model is particularly suitable for growth curves without lag phase (Figure 13). Y_0, Y_{max}, Y(t) are the bacterial population, **in natural logarithm of bacteria counts**, at initial, maximum, and time t. μ_{max} is the specific growth rate.

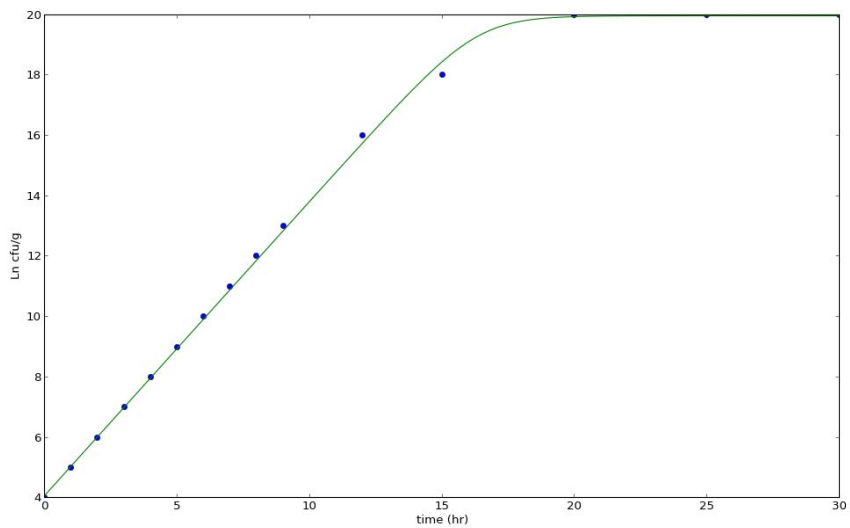

Figure 13. Growth curve without lag phase.

2. Reduced Huang Model (Huang, 2008)

Equation:

$$\boldsymbol{Y(t) = Y_0 + \mu_{max}\left[t + \frac{1}{4}ln\left(\frac{1+e^{-4(t-\lambda)}}{1+e^{4\lambda}}\right)\right]} \qquad \text{Eq. 2}$$

This model is a special case of the full Huang model, particularly suitable for growth curves that do not reach stationary phases (Figure 14). Y_0, Y_{max}, Y(t) are the bacterial population, **in natural logarithm of bacteria counts**, at initial, maximum, and time t. μ_{max} is the specific growth rate. λ is the lag phase duration.

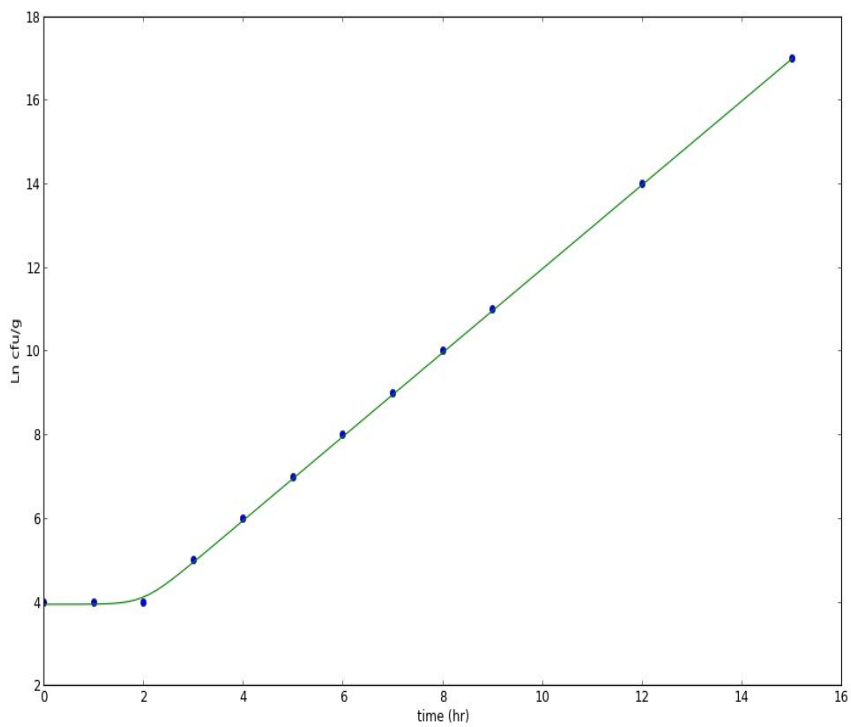

Figure 14. Reduced Huang Model.

3. Reduced Baranyi model (Baranyi and Roberts, 1995)

Equation:

$$\boldsymbol{Y(t) = Y_0 + \mu_{max}t + ln\left(e^{-\mu_{max}t} + e^{-h_0} - e^{-\mu_{max}t-h_0}\right)} \qquad \textbf{Eq. 3}$$

This is a special case of the full Baranyi model (Figure 15). Y_0, Y_{max}, Y(t) are the bacterial population, **in natural logarithm of bacteria counts**, at initial, maximum, and time t. μ_{max} is the specific growth rate. h_0 is the physiological state of the microorganism under consideration.

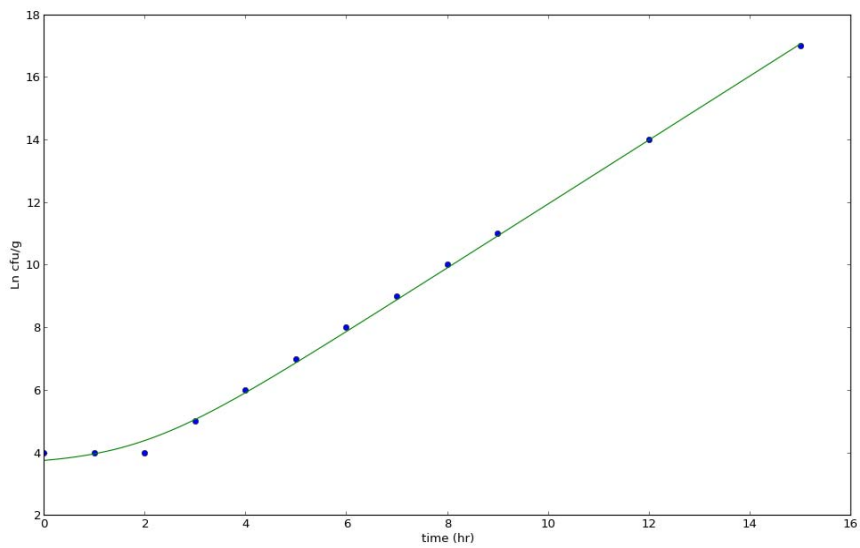

Figure 15. Reduced Baranyi Model.

4. Two-Phase Linear Growth model (Buchanan, Whiting, and Damert, 1997)

This model implements the concept original proposed by Buchanan, Whiting, and Damert (1997). The model can be expressed as

$$\boldsymbol{y = y_0, if\ t < lag}$$
$$\boldsymbol{y = y_0 + k(t - lag), if\ t \geq lag} \qquad \text{Eq. 4}$$

For this model, either log10 or Ln bacterial counts can be used.

Group 2. Full growth Models

1. Huang model (2008, 2013)

Equation:

$$Y(t) = Y_0 + Y_{max} - ln\{e^{Y_0} + [e^{Y_{max}} - e^{Y_0}]e^{-\mu_{max}B(t)}\} \qquad \text{Eq. 5}$$

$$B(t) = t + \frac{1}{\alpha} ln \frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{\alpha\lambda}}$$

This equation is the full Huang model (Figure 16). It is especially suitable for growth curves with distinct lag, exponential, and stationary phases. Y_0, Y_{max}, Y(t) are the bacterial population, **in natural logarithm of bacteria counts**, at initial, maximum, and time t. μ_{max} is the specific growth rate. λ is the lag phase duration. The lag phase transition coefficient α is 4.

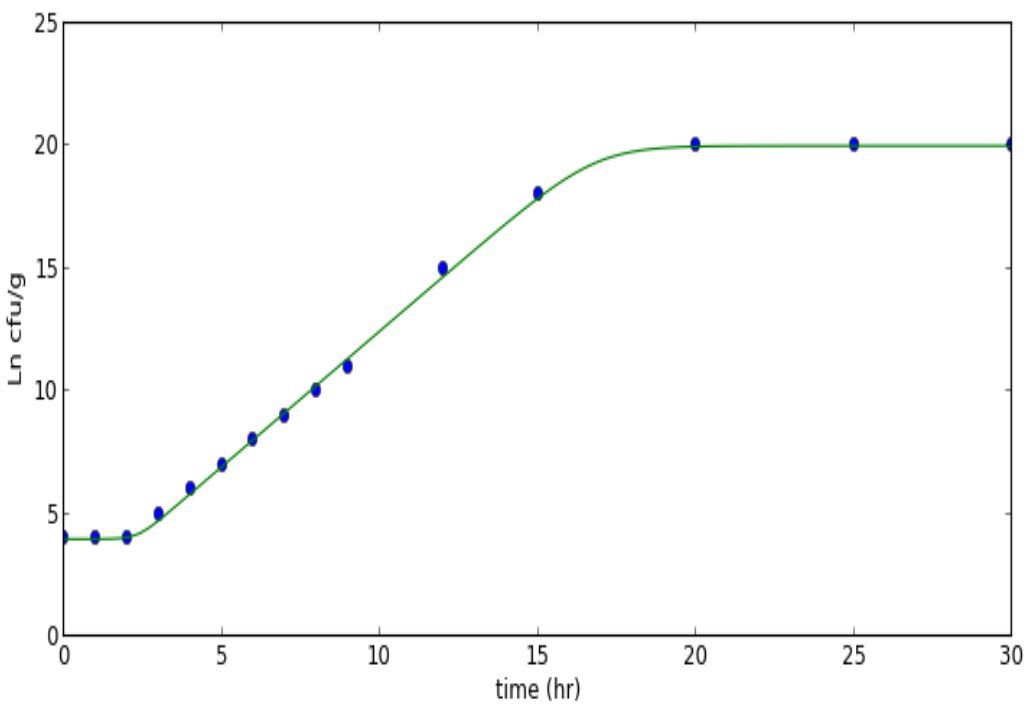

Figure 16. Full Huang model.

2. Baranyi model (Baranyi and Roberts, 1995)

Equation:

$$Y(t) = Y_0 + \mu_{\max} A(t) - \ln\left\{1 + \frac{\exp[\mu_{\max} A(t)] - 1}{\exp(Y_{\max} - Y_0)}\right\} \qquad \text{Eq. 6}$$

$$A(t) = t + \frac{1}{\mu_{\max}} \ln[\exp(-\mu_{\max} t) + \exp(-h_0) - \exp(-\mu_{\max} t - h_0)].$$

This is the full Baranyi model (Figure 17). Y_0, Y_{max}, Y(t) are the bacterial population, **in natural logarithm of bacteria counts**, at initial, maximum, and time t. μ_{max} is the specific growth rate. h_0 is the physiological state of the microorganism under consideration.

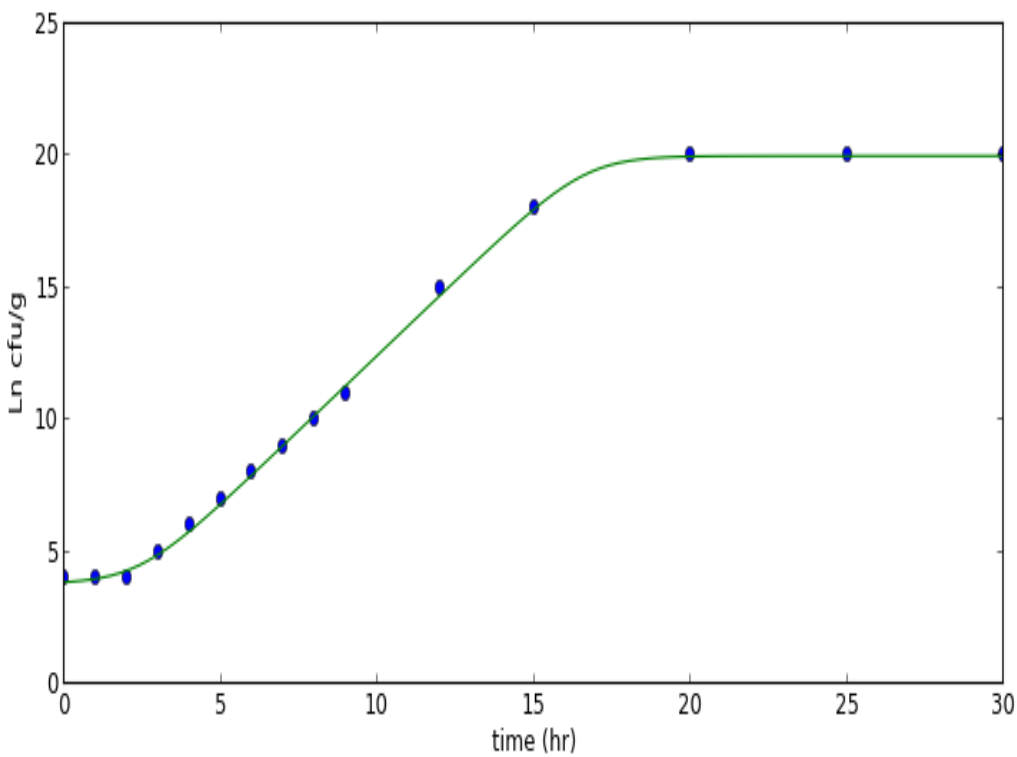

Figure 17. Full Baranyi model.

3. Re-parameterized Gompertz model (Zwietering, Jongenburger, Rombouts, and van't Riet, 1990)
Equation:

$$\boldsymbol{Y(t) = Y_0 + (Y_{max} - Y_0)exp\left\{-exp\left[\frac{\mu_{max}e}{Y_{max}-Y_0}(\lambda - t) + 1\right]\right\}} \qquad \text{Eq. 7}$$

This is an empirical model (Figure 18). Y_0, Y_{max}, Y(t) are the bacterial population, **in natural logarithm of bacteria counts**, at initial, maximum, and time t. μ_{max} is the specific growth rate. λ is the lag phase duration.

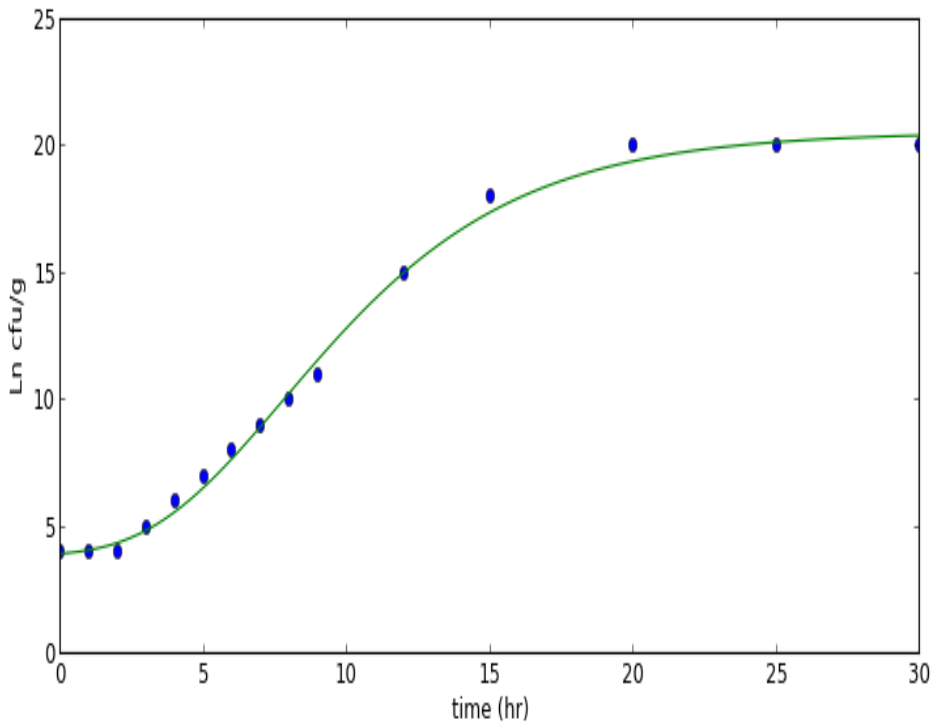

Figure 18. Reparamerized Gompertz model.

4. Three-Phase Linear Model (Buchanan, Whiting, and Damert, 1997)

This model implements the concept original proposed by Buchanan, Whiting, and Damert (1997). The model can be expressed as

$$\boldsymbol{y = y_0, if\ t < lag}$$
$$\boldsymbol{y = y_0 + k(t - lag), if\ lag \le t < t_{max}} \qquad \textbf{Eq. 8}$$
$$\boldsymbol{y = y_{max}, if\ t \ge t_{max}}$$

t_{max} is the time at which $y = y_{max}$.

For this model, either log10 or Ln bacterial counts can be used.

Group 3. Survival Models

1. Linear model

Equation:

$$y(t) = y_0 - \frac{1}{D}t \qquad \text{Eq. 9}$$

$$log(D) = log(D_0) - \frac{1}{z}T \qquad \text{Eq. 10}$$

This model is for determining linear thermal inactivation kinetics. y(t) and y_0 can be bacterial counts in **logarithms of base 10**; D is the thermal death time under a constant temperature; T is temperature; t is heating time under a constant temperature; z defines the effect of temperature on log(D).

2. Reparameterized Gompertz survival model (Huang, 2009)

Equation:

$$y(t) = y_0\left\{1 - exp\left[-exp\left(-\frac{\mu_{max}e}{y_0}(t-\lambda)+1\right)\right]\right\} \qquad \text{Eq. 11}$$

In this equation (Figure 19), y_0 and y are initial and real time bacterial counts (log10); μ_{max} is the maximum inactivation rate; λ is the initial lag phase; t is heating time under a constant temperature; e is 2.718.

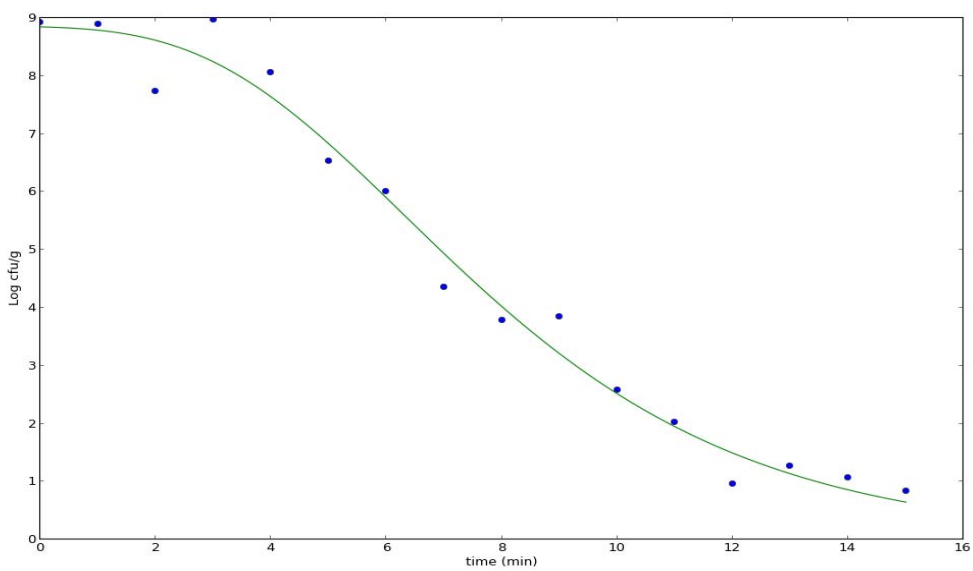

Figure 19. Reparamerized Gompertz survival model.

3. Weibull model (Huang, 2009)

Equation:

$$\boldsymbol{y(t) = y_0 - kt^{\alpha}} \qquad \text{Eq. 12}$$

In the Weibull equation (Figures 20 and 21), y_0 and y are initial and real time bacterial counts (log10); α can be > 1, = 1, or < 1, which determines the shape of the curves.

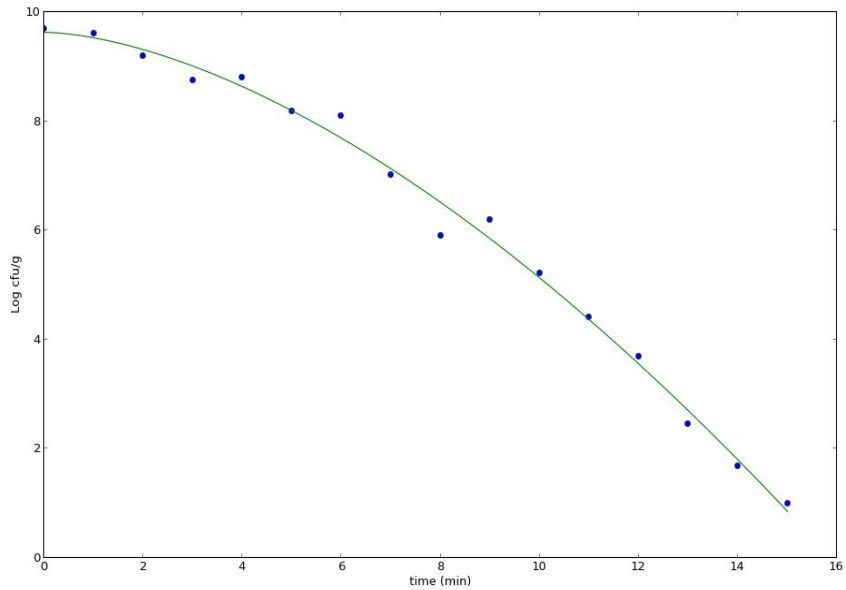

Figure 20. Weibull model (α > 1).

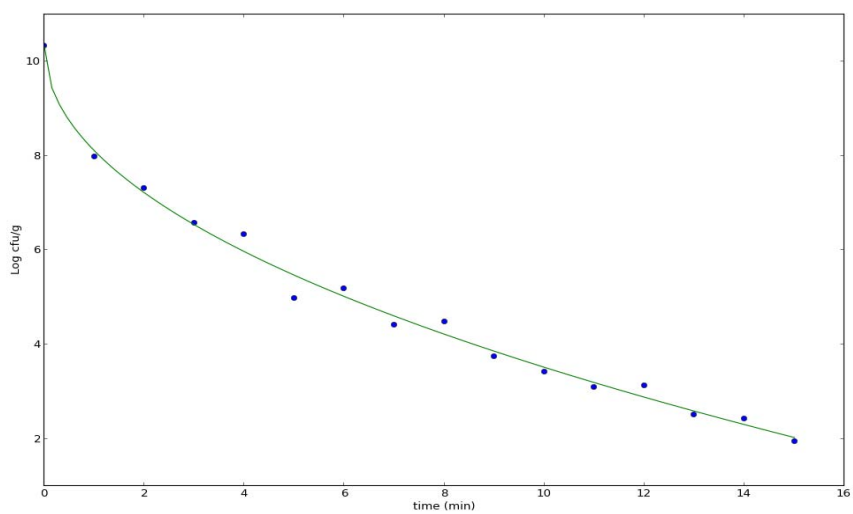

Figure 21. Weibull model (α < 1).

Group 4. Secondary models – effect of temperature on growth rate

1. Ratkowsky square-root model

1.1 Suboptimal Ratkowsky square-root model (Ratkowsky et al., 1983)

Equation:

$$\sqrt{\boldsymbol{\mu}} = \boldsymbol{a}(\boldsymbol{T} - \boldsymbol{T_0}) \qquad \text{Eq. 13}$$

In this equation (Figure 22), μ is the growth rate (time^{-1}); a is a coefficient; T is temperature; T_0 is the nominal minimum temperature. T_0 is usually not the biological minimum growth temperature.

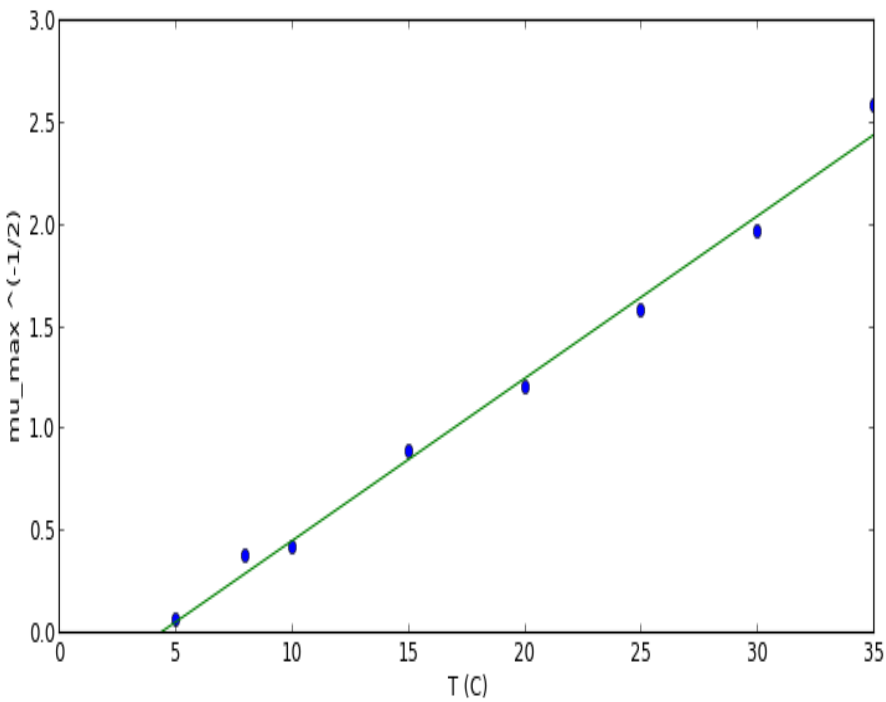

Figure 22. Sub-optimal Ratkowsky square-root model.

1.2 Full-temperature range Ratkowsky square-root model (Ratkowsky et al., 1983)

Equation:

$$\sqrt{\mu} = a(T - T_0)\left[1 - e^{b(T - T_{max})}\right] \qquad \text{Eq. 14}$$

In this equation (Figure 23), μ is the growth rate (time^{-1}); a and b are coefficients; T is temperature; T_0 is the nominal/notational minimum temperature; T_{max} is the estimated maximum growth temperature.

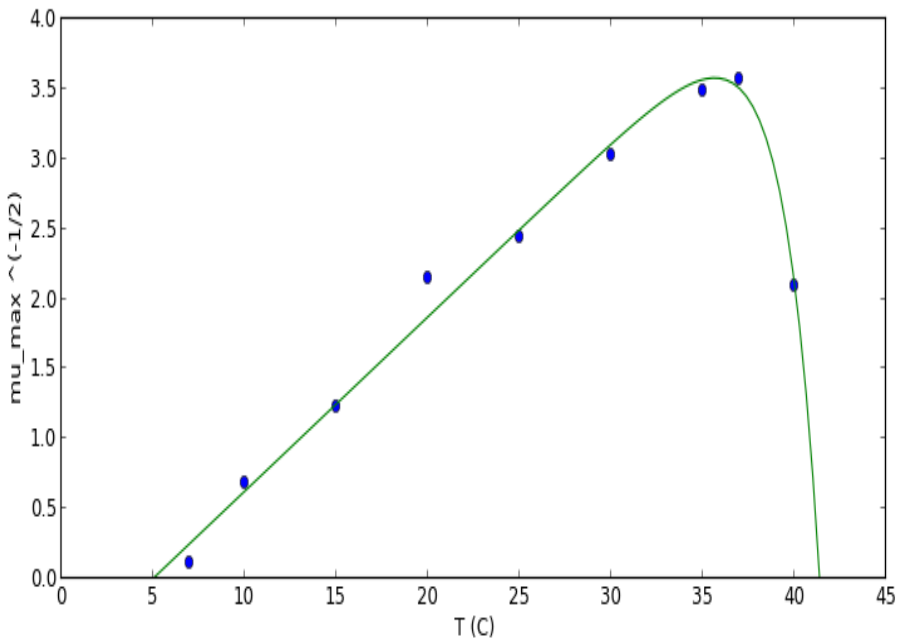

Figure 23. Full-temperature range Ratkowsky square-root model.

2. Huang square-root model

2.1 Suboptimal Huang square-root model (Huang, Hwang, and Phillips, 2011a)

Equation

$$\sqrt{\mu} = a(T - T_{min})^{0.75} \qquad \text{Eq. 15}$$

In this equation (Figure 24), μ is the growth rate (time^{-1}); a is a coefficient; T is temperature; T_{min} is the estimated minimum temperature.

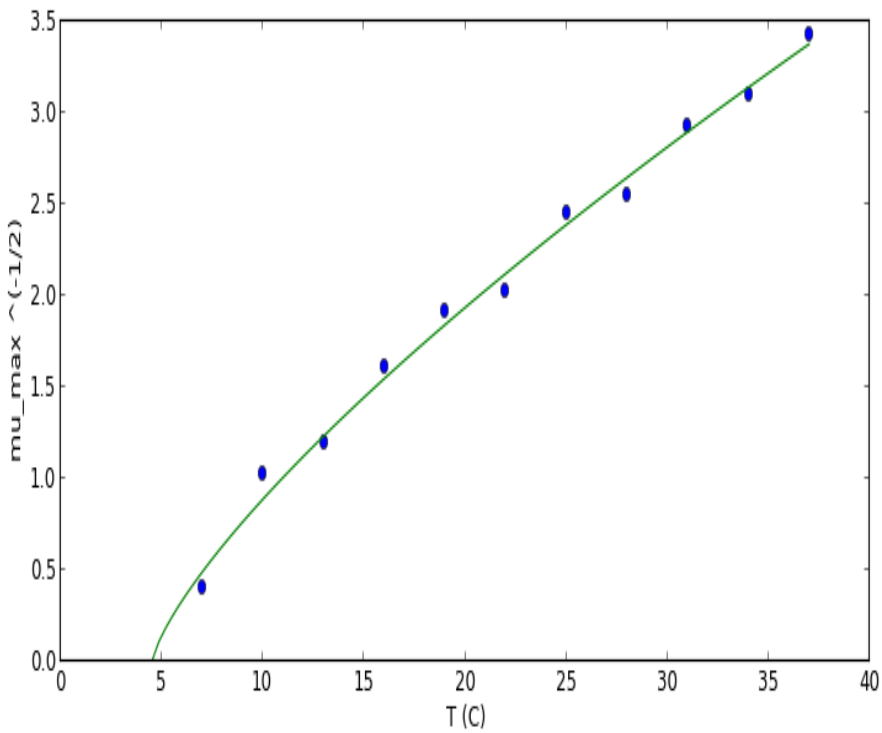

Figure 24. Sub-optimal Huang square-root model.

2.2 Suboptimal Huang square-root model (Huang, Hwang, and Phillips, 2011a)

Equation:

$$\sqrt{\mu} = a(T - T_{min})^{0.75}\left[1 - e^{b(T - T_{max})}\right] \qquad \text{Eq. 16}$$

In this equation (Figure 25), μ is the growth rate (time^{-1}); a and b are coefficients; T is temperature; T_{min} is the nominal/notational minimum temperature; T_{max} is the estimated maximum growth temperature.

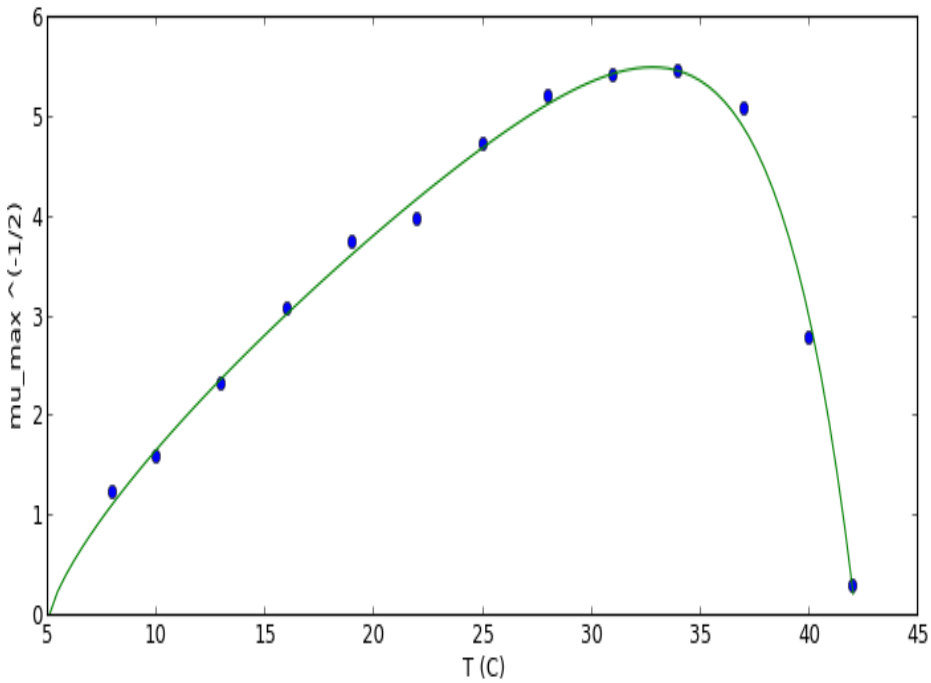

Figure 25. Full-temperature range Huang square-root model.

3. Cardinal model (Rosso, Lobry, and Flandrois, 1993)

Equation:

$$\boldsymbol{\mu_{max}} = \frac{\boldsymbol{\mu_{opt}(T-T_{max})(T-T_{min})^2}}{\boldsymbol{[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}-T_{min}-2T)](T_{opt}-T_{min})}} \qquad \text{Eq. 17}$$

In this equation (Figure 26), μ_{max} is the maximum growth rate at each temperature (T); μ_{opt} is the optimum growth rate at the optimum temperature (T_{opt}); T_{min} and T_{max} are the minimum and maximum growth temperature. The Cardinal model is only suitable for full-temperature range.

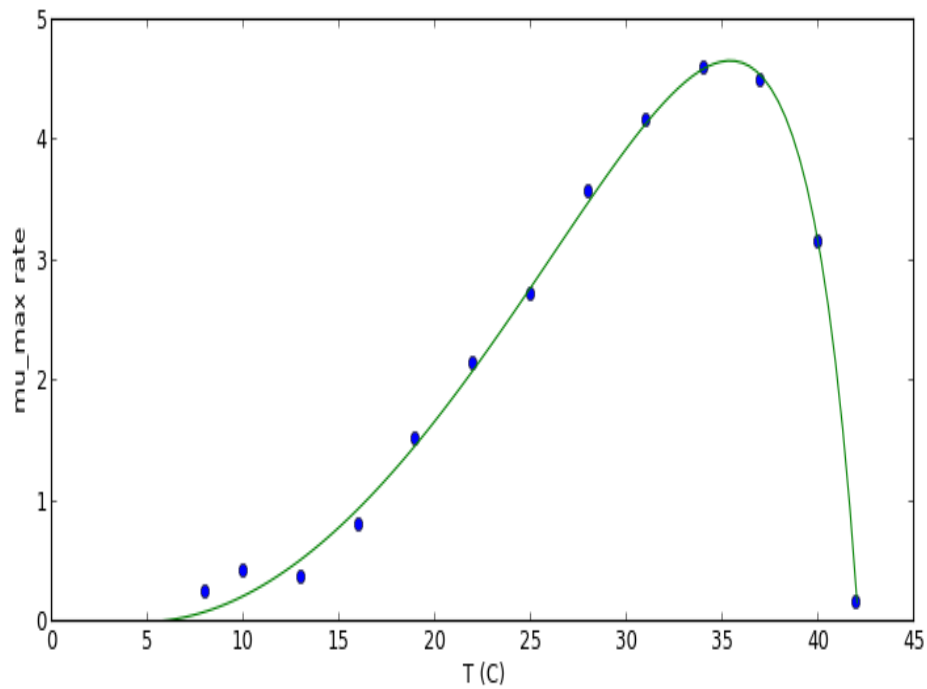

Figure 26. Cardinal model.

4. Arrhenius-type model (Huang, Hwang, and Phillips, 2011b)

4.1 Sub-optimal Arrhenius-type model

Equation:

$$\boldsymbol{\mu_{max} = a(T + 273.15)exp\left\{-\left[\frac{\Delta G'}{R(T+273.15)}\right]^{n}\right\}} \qquad \text{Eq. 18}$$

In this equation (Figure 27), R is gas constant (8.134 J/mol), ΔG' is a type of kinetic energy related to bacterial growth, a and n are coefficients; T is temperature in Celsius.

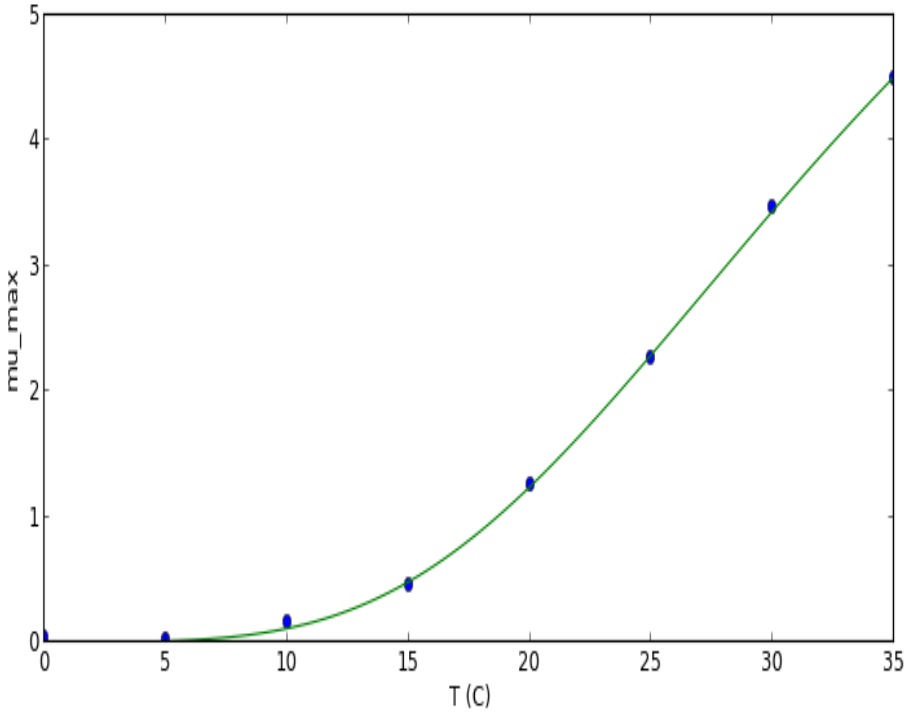

Figure 27. Sub-optimal temperature range Arrhenius-type model

4.2 Full-temperature range Arrhenius-type model (Huang, Hwang, and Phillips, 2011b)

Equation:

$$\boldsymbol{\mu_{max} = a(T + 273.15)exp\left\{-\left[\frac{\Delta G'}{R(T+273.15)}\right]^{n}\right\}\left[1 - e^{b(T-T_{max})}\right]} \qquad \text{Eq. 19}$$

In this equation (Figure 28), R is gas constant (8.134 J/mol), ΔG' is a type of kinetic energy related to bacterial growth, a, b and n are coefficients; T is temperature in Celsius; T_{max} is the maximum growth temperature.

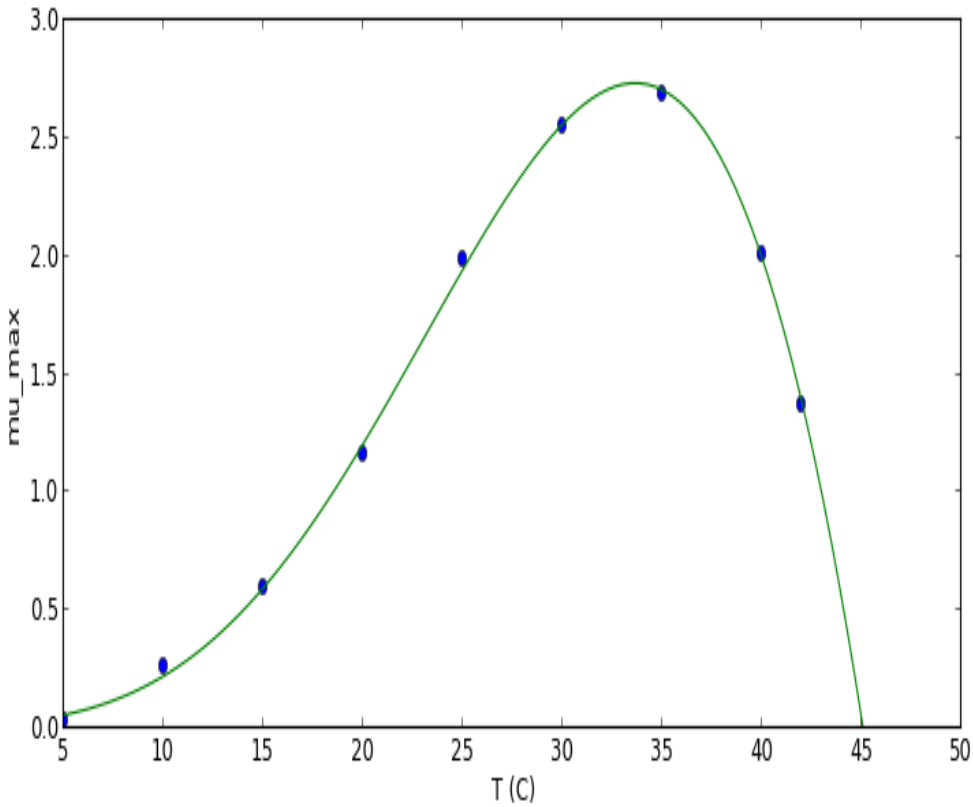

Figure 28. Full-temperature range Arrhenius-type model.

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