

GROWTH PREDICTOR

User guide

<https://skandamis.shinyapps.io/Microbial-Growth-Predictor-Dashboard/>

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1. General output

The main starting page of the tool looks as follows:

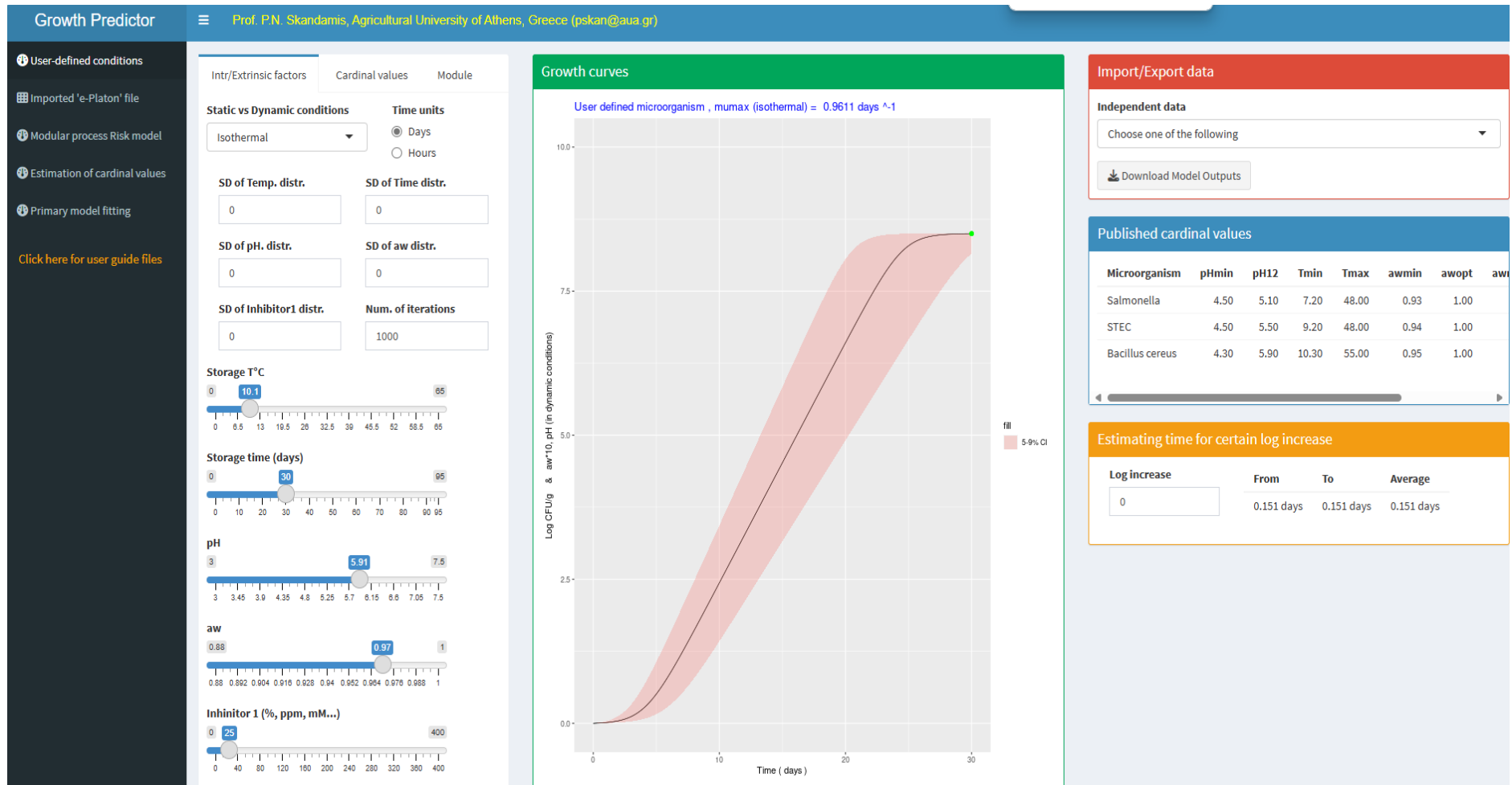


Figure 1. Starting page of the software tool.

General description

On the left panel of the main form, there are 3 modules:

- **“User-defined conditions”**: the user may simulate microbial growth of any microorganism, under isothermal (static) or non-isothermal (dynamic) conditions, with user-defined cardinal values of T , pH , a_w and up to 6 inhibitors, most of them introduced either as single values (deterministically), or as normal distributions (stochastically sampled with *Monte Carlo* simulation), or selected from a built-in database of cardinal values for different microorganism, in the tool. When selecting cardinal values from the built-in dbase, there is still the option to describe the inter-strain variability in the cardinal values of the organism, by typing the standard deviation (SD) of the normal distribution describing the cardinal values, since this information is not included in the built-in dbase. The lag time can also be introduced deterministically or stochastically as normal distribution of the *Work-to-be-done* (ho). The model outputs can be downloaded in an XL file and saved on a local disc.
- **“Imported ‘e-Platon’ file”**: the user imports data from the food safety dashboard ‘e-Platon’ ([Microsoft Power BI](#)), in the form of an XL file that contains product name, the values of pH , a_w and the concentration of *nitrites* per product, along with the cardinal values for *L. monocytogenes* strains, or of any other pathogen stored in the **e-Platon** database. Once the XL file is uploaded, growth simulations may be obtained isothermally for different temperatures and storage times, or under non-isothermal (dynamic) conditions of time and temperature. The user may select specific products as numbers 1, 2, 3, etc., from the imported file and perform the same operations as in the “User-defined conditions” module. In particular, the user may define the cardinal values, deterministically or as normal distributions and also assess the log reductions caused by a heating step at the end of storage. The lag time may be introduced deterministically or stochastically, as in the “User-defined conditions” module. The model outputs can be downloaded in an XL file and saved on a local disc.
- **“Modular process model”**: this is a modular process Quantitative Microbial Risk Assessment (QMRA) model, where the food chain is split in 4 stages, namely “Farm to end of processing”, “End of processing to retail”, “Retail” and “Domestic storage”. The user may introduce deterministically or stochastically the storage time and temperature, or as multiple time-T profiles, contamination or re-contamination levels of a pathogen, the reduction due to cooking or processing, the product characteristics (pH , a_w and only 1 inhibitor) and the corresponding cardinal values of the target pathogen. The impact of additional inhibitors should be described via the μ_{ref} value in the presence of the inhibitors, based on the “User-defined conditions” tab. The final output based on these inputs, is a distribution of dose at consumption and the log of Probability of illness, according to the prevalence and the serving size. The user may select one of the following three dose-response models: (i) *Listeria monocytogenes* FDA model (FDA 2014) for susceptible population, (ii) the same model for non-susceptible population and (iii) STEC model (Cassin et al. 2000). The numerical QMRA outputs include the average probability of illness per serving (P_{ill}) and the expected (predicted) number of annual cases, after setting the total number of annual servings. All model outputs can be downloaded in an XL file and saved on the hard disc at a path specified by the user. The output file includes model parameters (parameter and types of distributions, model and microorganism characteristics, cardinal values,

etc.), the iterated values of input and output distributions and selected metrics of the exposure and P_{III} /predicted number of cases.

The screenshot shows the 'User-defined conditions' module interface. It has three tabs: 'Intr/Extrinsic factors' (selected), 'Cardinal values', and 'Module'. The 'Intr/Extrinsic factors' tab contains the following sections:

- Static vs Dynamic conditions:** A dropdown menu set to 'Isothermal'.
- Time units:** Radio buttons for 'Days' (selected) and 'Hours'.
- SD of Temp. distr.:** Input field with value 0.85.
- SD of Time distr.:** Input field with value 0.
- SD of pH. distr.:** Input field with value 0.
- SD of aw distr.:** Input field with value 0.003.
- SD of Inhibitor1 distr.:** Input field with value 0.
- Num. of iterations:** Input field with value 1000.
- Storage T°C:** A slider ranging from 0 to 65, with a value of 4 selected.
- Storage time (days):** A slider ranging from 0 to 95, with a value of 30 selected.
- pH:** A slider ranging from 3 to 7.5, with a value of 5.31 selected.
- aw:** A slider ranging from 0.88 to 1, with a value of 0.97 selected.
- Inhibitor 1 (% , ppm, mM...):** A slider ranging from 0 to 400, with a value of 25 selected.
- Add more inhibitors:** A button.
- Initial contamination (Log CFU/g or ml):** Input field with value 0.
- Maximum population density (Log CFU/g or ml):** Input field with value 8.5.
- Work-To-Be-Done (h0: 0.1 to infinity):** Input field with value 0.01.
- sd h0 (stochastic lag):** Input field with value 0.

Annotations on the right side of the image point to specific features:

- A dashed arrow points from the 'Module' tab to a box: 'Tab for defining the heat inactivation step after growth'.
- A dashed arrow points from the 'Cardinal values' tab to a box: 'Tab for setting the cardinal values for the secondary'.
- A dashed arrow points from the 'Static vs Dynamic conditions' dropdown to a box: 'Selecting isothermal/non-isothermal conditions (at this tab)'.
- An orange bracket groups the 'Time units', 'SD of Temp. distr.', 'SD of Time distr.', 'SD of pH. distr.', 'SD of aw distr.', 'SD of Inhibitor1 distr.', and 'Num. of iterations' fields, pointing to a box: 'Setting the time and the independent variables controlling microbial growth. 1. **Temperature, pH, aw, inhibitor** and time can also be introduced as **normal distributions** 2. Defining the number of iterations to be considered when any of the variables is introduced stochastically as normal distribution (i.e., SD>0) 3. **"Add more inhibitors"**, enables the addition of values for 5 more inhibitors (phenolics, CO₂, organic acids, etc.)
- A green bracket groups the 'Initial contamination' and 'Maximum population density' fields, pointing to a box: 'Setting the initial and maximum (at stationary phase) contamination level'.
- A blue bracket groups the 'Work-To-Be-Done' and 'sd h0' fields, pointing to a box: 'Setting the lag time properties'.

Figure 2. Main side panel of "User-defined conditions" module (top-left on the black side-panel).

2. Module “Intrinsic/Extrinsic factors”

These factors characterize the product, storage and packaging conditions, which, together with the time, control microbial growth, according to the “**multiple hurdle theory**”. The secondary model used to estimate the μ_{max} is a gamma model with or without interactions (ξ) (**a user-enabled option, available at the “Cardinal value” tab**), including terms for T , pH , a_w , and one inhibitor, e.g., nitrites (by default, $n1=1$ and $n2=2$) of the following form (Mejlholm et al. 2010):

$$\begin{aligned}\mu_{max} &= \mu_{ref} \left(\frac{T-T_{min}}{T_{ref}-T_{min}} \right)^2 (1 - 10^{pH_{min}-pH}) \left(\frac{a_w-a_{wmin}}{1-a_{wmin}} \right) \left[1 - \left(\frac{C_i}{MIC} \right)^{n1} \right]^{n2} = \\ &= \mu_{ref} \left(\frac{T-T_{min}}{T_{ref}-T_{min}} \right)^2 (1 - 10^{pH_{min}-pH}) \left(\frac{a_w-a_{wmin}}{1-a_{wmin}} \right) \left(1 - \frac{NO_3}{MIC} \right)^2 \xi\end{aligned}\quad \text{Equation (1)}$$

$$\xi = \begin{cases} 1 & , \psi \leq 0.5 \\ 2(1 - \psi) & , 0.5 < \psi < 1 \\ 0 & , \psi \geq 1 \end{cases}\quad \text{Equation (2)}$$

$$\text{where, } \psi = \sum_i \frac{\varphi_{e_i}}{2 \prod_{j \neq i} (1 - \varphi_{e_j})}\quad \text{Equation (3)}$$

$$\varphi(T) = \left[1 - \left(\frac{T-T_{min}}{T_{ref}-T_{min}} \right) \right]^2\quad \text{Equation (4)}$$

$$\varphi(a_w) = \left(1 - \sqrt{\frac{a_w-a_{wmin}}{a_{wopt}-a_{wmin}}} \right)^2 \quad \text{default } a_{wopt} \text{ is set at 1}\quad \text{Equation (5)}$$

$$\varphi(pH) = \left(1 - \sqrt{1 - 10^{pH_{min}-pH}} \right)^2\quad \text{Equation (6)}$$

$$\varphi(c_i) = \left(1 - \sqrt{1 - \frac{c_i}{MIC_i}} \right)^2\quad \text{Equation (7)}$$

Equation 7 applies to nitrites, as well as CO_2 (or O_2) and phenolic compounds, when they are selected as additional inhibitors (see further down),

$$\text{and } \varphi(OA_1, OA_2, \dots, OA_i) = (1 - OA_1 \cdot OA_2 \cdot \dots \cdot OA_i)^2 = \left\{ 1 - \left[\left(1 - \sqrt{\frac{OA_{U,1}}{MIC_{U,1}}} \right) \left(1 - \sqrt{\frac{OA_{U,2}}{MIC_{U,2}}} \right) \left(1 - \sqrt{\frac{OA_{U,3}}{MIC_{U,3}}} \right) \dots \left(1 - \sqrt{\frac{OA_{U,i}}{MIC_{U,i}}} \right) \right] \right\}^2 \quad \text{Equation (8)}$$

for the mixture of organic acids, applicable when additional inhibitors are selected (see further down, Pages 10-11). Each organic acid term may be introduced with its own exponents.

- C_i : is the concentration of the inhibitor in *ppm*, *mM* or else. The user may choose to enter the concentration in the water phase or the total in food. Care should be taken for consistency with the conditions, which were used for estimation of μ_{ref} , experimentally.
- MIC : is the Minimum Inhibitory Concentration of the inhibitor.

- T_{ref} is the reference temperature, at which, the μ_{ref} has been calculated. When Rosso model is selected for the temperature gamma term, T_{ref} becomes T_{opt} and $\mu_{ref}=\mu_{opt}$.

The input values for the independent variables (T , a_w , pH , C_i) under **isothermal** (static) conditions are set in the same tab via the following sliders (Figure 3):

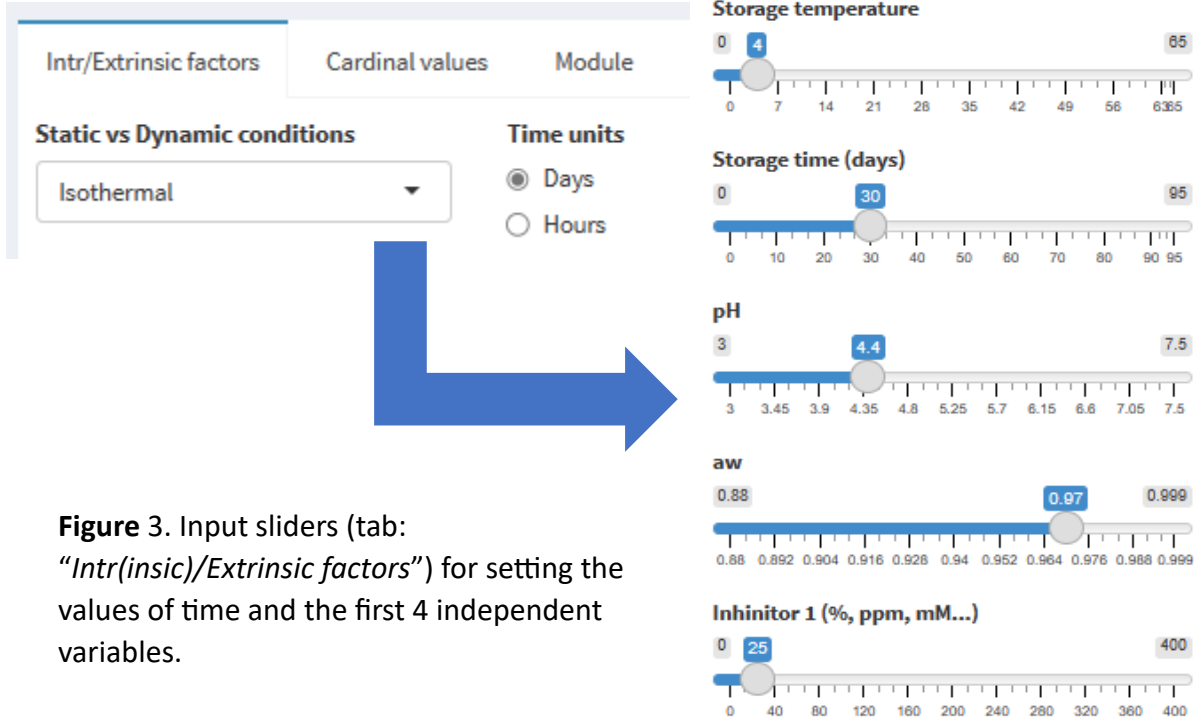


Figure 3. Input sliders (tab: “Intr(insic)/Extrinsic factors”) for setting the values of time and the first 4 independent variables.

Of the (independent) input values, **temperature** and **time** are the only ones that can be introduced both deterministically or stochastically as normal distribution $N(\text{mean}=\text{slider input}, SD=\text{typed input})$.

The cardinal values of the input (explanatory) variables are defined at the tab “Cardinal values”. They include: pH_{min} , a_{wmin} , MIC , the exponents 1 ($n1$) and 2 ($n2$) of each inhibitor, μ_{ref} , a_{wref} and T_{ref} (Figure 4). The boxes for T_{max} , a_{wmax} , pH_{opt} and pH_{max} are enabled when the relevant Rosso-type terms (equation 9) are selected for T , a_w and pH , respectively, in the “Cardinal values” tab (Figure 4). Then, T_{ref} and a_{wopt} represent T_{opt} and a_{wopt} , whereas μ_{ref} becomes μ_{opt} .

$$CM_n(X) = \begin{cases} 0, & X \leq X_{min} \\ \frac{(X - X_{max})(X - X_{min})^n}{(X_{opt} - X_{min})^{n-1} \{ (X_{opt} - X_{min})(X - X_{opt}) - (X_{opt} - X_{max}) [(n-1)X_{opt} + X_{min} - nX] \}}, & X_{min} < X < X_{max} \\ 0, & X \geq X_{max} \end{cases}$$

Equation (9): $n=2$ for T , a_w ; $n=1$ for pH .

Similarly, the input box for the parameter pH_{12} , which represents the pH at which μ_{max} equals to half the value of μ_{opt} in the gamma term introduced by (Aryani et al. 2015; equation 10) is enabled when the respective model has selected from the icon: ☐ pH_{12} model still in the “Cardinal values” tab:
for pH

$$\gamma(pH) = \frac{1 - 2^{\frac{(pH - pH_{min})}{(pH_{min} - pH_{1/2})}}}{1 - 2^{\frac{(pH_{ref} - pH_{min})}{(pH_{min} - pH_{1/2})}}}$$

where pH_{ref} = a reference pH Equation (10)

The screenshot shows the 'Cardinal values' tab of a software interface. It contains several input fields for cardinal values. A blue arrow points from the 'Rosso model for T°C' checkbox to a zoomed-in inset of the 'Deterministic vs stochastic growth limits' section.

Cardinal values tab:

- Intr/Extrinsic factors:** (tab)
- Cardinal values:** (active tab)
- Module:** (tab)
- Deterministic vs stochastic growth limits:**
 - Fixed value (dropdown)
 - ☐ Rosso model for T°C

Input fields:

- Tmin:** -0.92
- Tmax (when Rosso model is enabled):** 48
- pHmin:** 4.4
- awmin:** 0.915
- MIC of inhibitor 1:** 332
- Exponent 1 of inhibitor 1 term:** 1
- Exponent 2 of inhibitor 1 term:** 2
- Tref (or Topt):** 25
- muref (or muopt) 1/h:** 0.413

Inset (Deterministic vs stochastic growth limits):

- ☒ Rosso model for T°C
- Tmin:** -0.92
- Tmax (when Rosso model is enabled):** 48

Figure 4. Setting the cardinal values for the secondary model in the “Cardinal values” tab. The box of T_{max} is enabled when Rosso model for T°C is selected.

2.1. Cardinal values reflecting strain-variability or built-in databases

Apart from assigning a single value, the cardinal values for the minimum T , pH , a_w , and the *Minimum Inhibitory Concentration*-MIC of 1 inhibitor can be introduced as normal distributions with mean and SD defined in the proper numeric input boxes (Figure 5), or they can be directly extracted by a built-in database (Figure 6). Stochastic cardinal values may reflect inter-strain

variability in the growth limits. Growth simulation under variable conditions (T , pH , a_w and inhibitor), as well as time and cardinal values is performed with MC simulation for user-defined number of iterations.

Intr/Extrinsic factors
Cardinal values
Module

Deterministic vs stochastic growth limits

Normal dist. ▼

☐ Rosso model for T°C

Average Tmin

-0.92

SD Tmin

0.5

Average Tmax (when Rosso model is enabled)

48

SD Tmax (when Rosso model is enabled)

0.5

Average pHmin

4.4

SD pHmin

0.1

Average awmin

0.915

SD awmin

0.001

Average MIC of inhibitor 1

332

SD MIC

10

The graph displays a growth curve over 40 days. The y-axis is log CFU/g (a_w, pH in dynamic conditions) ranging from 1.0 to 10.0. The x-axis is Time (days) ranging from 0 to 40. A solid black line represents the mean growth, and a shaded orange ribbon represents the 5-95% percentile range. A legend indicates '5-95 CI'.

The 2 boxes are enabled only when Rosso model for T°C is selected above. The same applies to the cardinal values of a_w and pH , i.e., a_{wopt} , a_{wmax} , pH_{12} , pH_{opt} , pH_{max} , etc.

Deterministic vs stochastic growth limits

Normal dist. ▼

☒ Rosso model for T°C

Average Tmin

-0.92

SD Tmin

0.5

Average Tmax (when Rosso model is enabled)

48

SD Tmax (when Rosso model is enabled)

0.5

Figure 5. Form for defining the cardinal values as normal distributions (“Normal dist.”), e.g., representing strain variability. The orange ribbon in the graph above and below the growth curve shows the 5-95% percentile range of growth simulation when cardinal values are defined as normal distributions (see further down in 2.4 and 2.5 the detailed description of graphical outputs).

When the options “Fixed value” or “Normal dist.” are selected, the cardinal values are user-defined according to the relevant numeric input boxes. In contrast, when the option “Pick from dbase” is selected, then the average cardinal values are constrained by those stored in the system dbase. The user-defined values are restored when the “Fixed value” or “Normal dist.” are re-selected.

IMPORTANT NOTE: *If the cardinal values are changed under “Fixed value” or “Normal dist.”, then, when shifting between the two, the graphs may differ due to differences in the cardinal values corresponding to each session. The user needs to update the values for agreement.*

Intr/Extrinsic factors

Cardinal values

Module

Deterministic vs stochastic growth limits

Pick from dbase

☐ Rosso model for T°C

Microorganism

Salmonella

Salmonella

STEC

Bacillus cereus

SD Tmax

0

SD pHmin

0

SD awmin

0

SD MIC

0

Add more inhibitors

Published cardinal values

Microorganism	pHmin	Tmin	Tmax	awmin	MIC_Inhibitor_1
Salmonella	4.50	7.20	48.00	0.93	332.00
STEC	4.50	9.20	48.00	0.94	332.00
Bacillus cereus	4.30	10.30	55.00	0.95	332.00

Figure 6. Defining the cardinal values for T , pH , a_w and *inhibitor 1* for a given organism based on the existing tool dbase (“Pick from dbase”). To include more microorganisms in the dbase, please contact the administrator at pskan@aua.gr. Once this option is selected, the user cannot interfere with the average value of each cardinal value, but only with their *SD* (under “Normal dist.”).

By pressing

Add more inhibitors

 the user may define up to 5 more inhibitors, as multiplicative terms in the above gamma-type equation, with the following format:

$$\left[1 - \left(\frac{C_i}{MIC_i}\right)^{n1}\right]^{n2} \quad \text{Equation (11)}$$

The input values are introduced *via* the slider shown below (Figure 7). The names of the inhibitors are simply indicative, but consistent with the expected range of values in different foods they are intended to be used.

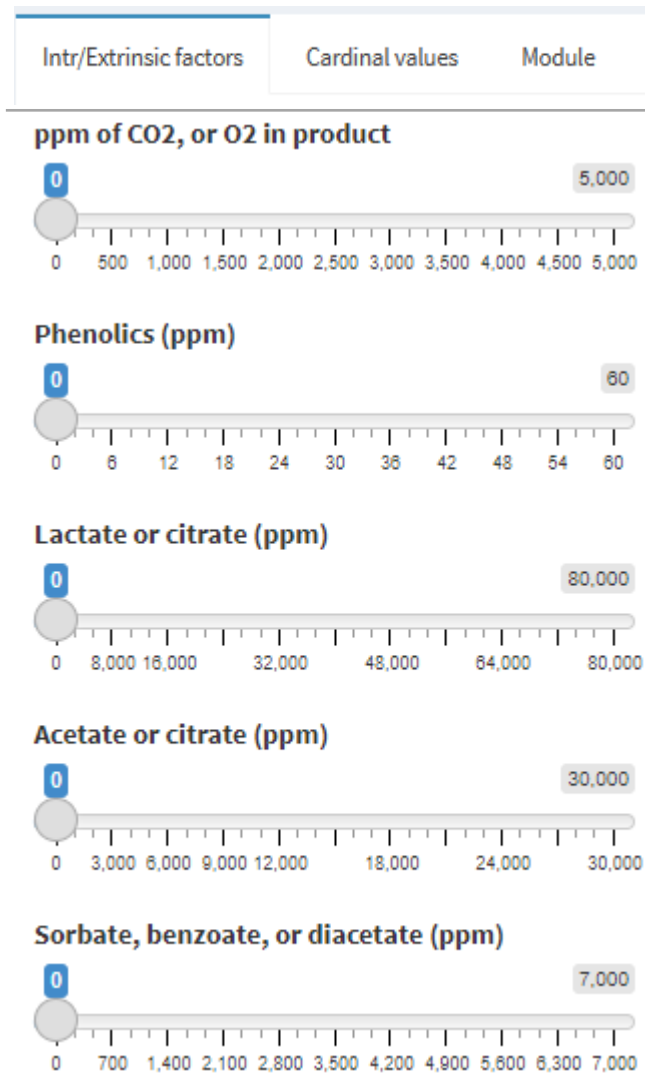


Figure 7. Input sliders (tab: "Intr(insic)/Extrinsic factors") for the level of each of the 5 extra inhibitors in foods.

Figure 8 displays input boxes for the 'Cardinal values' tab. The inputs are: MIC CO2, or O2 (3140), Exp. 1 of CO2 or O2 term (0.5), Exp. 2 of CO2 or O2 term (1), MIC Phenolics (20), Exp. 1 of phenolics term (1), Exp. 2 of phenolics term (1), MIC Lactate or Citrate (50000), Exp. 1 of Lact/Citr. term (1), Exp. 2 of Lact/Citr. term (1), MIC Acetate or Citrate (15000), Exp. 1 of Acet/Citr. term (0.5), Exp. 2 of Acet/Citr. term (1), MIC Sorbate, benzoate or diacetate (2500), Exp. 1 of Sorb/benz/DA term (0.5), and Exp. 2 of Sorb/benz/DA term (1).

Figure 8. Input boxes (tab: "Cardinal values") for the MIC and the exponents of each of the 5 extra inhibitors.

In particular:

- The 1st of the extra inhibitors could be the % of CO₂ or O₂ concentration in the package headspace that could receive values from 0 to 100.
- The 2nd inhibitor is the concentration of phenolic compounds associated with smoking processes, applicable, for example, in smoked RTE meat and seafood products.
- The 3rd and 4th inhibitor could represent lactate, or acetate acid, respectively. Alternatively any the 2 could represent citric acid and the other either lactate or acetate. The 5th inhibitor represents the levels of sorbate and benzoate. The user may ignore the proposed nomenclature and consider other substitutes of the default inhibitors, provided that their levels fit in the range of values allowed by the sliders.

The MIC_i and the 2 exponents of each inhibitor i ($n1$, $n2$ of equation 9), are set in the “*Cardinal values*”, similarly to the corresponding values of the first inhibitor (Figure 8).

2.2. Estimating the μ_{ref} for a product formulated with multiple inhibitors

The default values for μ_{ref} refer to the absence (value=0) of any of the 6 inhibitors. To estimate the theoretical μ_{ref} in a food with multiple inhibitors ($Prod_{ref}$), the user needs to adjust the values of the inhibitors to their intrinsic values in the food of concern at the default (25°C) or a new (user-defined) T_{ref} . Then, the old μ_{ref} in the relevant box at the ‘*cardinal values*’ tab should be replaced by the newly predicted μ_{max} that appears on top of the graphical output (Figure 9). This is now the expected μ_{ref} at the new reference product ($Prod_{ref}$) at T_{ref} and reflects the impact of the multiple inhibitors in the formulation, as described above.

To confirm that, the user may set all inhibitors to 0 value. The predicted μ_{max} would now be equal to the new μ_{ref} . As such, any value of the inhibitors from now on would equal the extra amount of inhibitors added to the product already formulated with inhibitors ($Prod_{ref}$).

This value could be used in the other modules of the tool (‘Import ‘*e-Platon*’ file’ and ‘*Modular Process model*’), where input levels for only 1 inhibitor are foreseen.

2.3. Microorganism related properties

To perform the growth simulation, the user needs to define the “*initial contamination (Log CFU/g or ml)*”, the “*Maximum population density (Log CFU/g or ml)*” and the “*Work-to-be-done*” (h_0 of the Baranyi model; Baranyi and Roberts 1994) that reflects the lag time (λ) of the organism. The complete form of the primary growth model used in the tool is:

$$\frac{dN}{dt} = \frac{q_t}{q_t+1} \mu_{\max} \left(1 - \left(\frac{N_t}{N_{\max}}\right)^m\right) N_t, \quad \text{where } q_t = \frac{P_t}{K_p} \quad \text{Equation (12)}$$

$$h_0 = \ln\left(1 + \frac{1}{q_0}\right) = -\ln(a_0) = \mu_{\max} \lambda \quad \text{Equation (13)}$$

where q_t is the physiological state of the microorganism, that depends on the cell ‘history’ inherited from their previous environments. It is defined as the ratio of a per cell critical product (P_t) that needs to be produced by the organism to exit the lag phase and the *Michaelis-Menten* constant K_p . P_t follows Monod kinetics. The variable a_0 represents the percentage of cells in the new environment that continue to grow with the μ_{\max} of the previous environment, i.e., without being affected by the shift to the new environment. As such, a_0 equal to 0 suggest no growth and 1, suggests no lag.

The lag time depends on h_0 and μ_{\max} according to equation 13, which represents the work-to-be-done by the microorganism to exit the lag phase. The default value for h_0 in the tool is the lowest acceptable one, i.e., 0.01, whereas it can receive values up to infinity. The higher the value of h_0 , the longer the lag time. h_0 can be also introduced as normal distribution with h_0 as mean value and SD defined by the user in the last box of the Int/Extrinsic factors (on the lefthand side below the sliders of the input variables – see below).

sd h_0 (stochastic lag)

2.4. Outputs under static conditions

The differential form of the Baranyi model (equation 12; Baranyi et al. 1995) is solved numerically with the *Rung-Kutta 4* method. The μ_{\max} at the user-defined conditions of T , pH , a_w and the levels of up to 6 *inhibitors*, is calculated by the gamma-type secondary model of equation 1, **with (default) or without interaction**, by unticking or ticking the box “including the χ_{si} Interaction term”, respectively, in the tabs “Cardinal values”.

If the “Rosso model” is selected in the “Cardinal values” tab (Figures 4 & 5), the temperature term of equation 1 is replaced by equation 9. **When Rosso gamma temperature term is enabled, then T_{ref} represents T_{opt} and μ_{ref} becomes μ_{opt} .** Likewise, when the “pH12” model is selected (can be co-selected with “Rosso model”), the gamma term for pH in equation 1 is replaced by equation 10 above (Aryani et al. 2015).

The simulation under static outputs without lag time looks like Figure 9. The user may select simulation time in days or hours by ticking the proper radio button on top right of the

“Intr/Extrinsic properties” tab. The same requirement holds also for dynamic conditions, as detailed further down in the document.

Intr/Extrinsic factors

Cardinal values

Module

Static vs Dynamic conditions

Isothermal

Time units

Days

Hours

When simulation is based on user-defined cardinal values as fixed value or normal distribution, the title is “*User defined microorganism*”. When cardinal values are picked up from the dbase, then the name of the organism appears at the top of the graph.

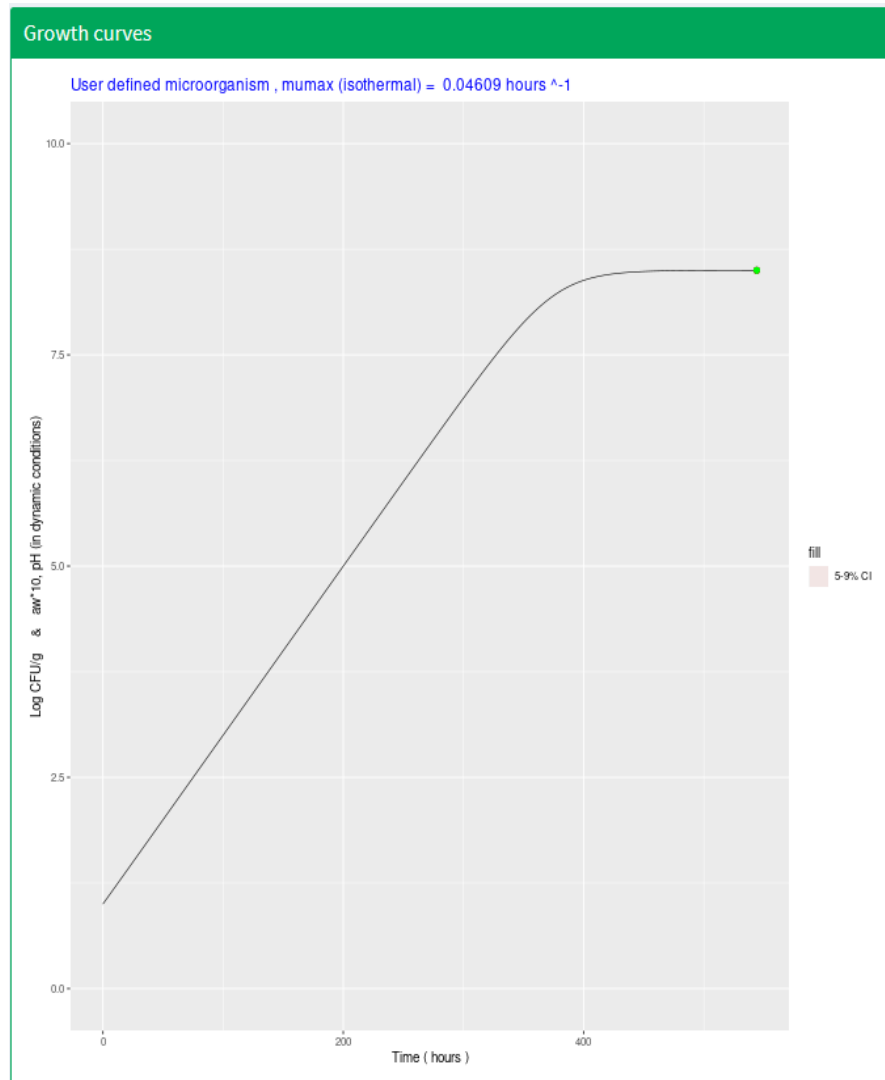


Figure 9. Growth simulation, without lag ($h_0=0.01$), initial contamination 1 Log CFU and maximum population density (at stationary phase) set at 8.5 Log CFU/g or ml. Simulation time = 544 h, $T=10^{\circ}\text{C}$, $\text{pH}=5.95$, $a_w=0.97$, Inhibitor 1=0 and default cardinal values (i.e., $T_{\min}=-0.92$, $\text{pH}_{\min}=4.4$, $a_{w\min}=0.915$, $T_{\text{ref}}=25^{\circ}\text{C}$, $\mu_{\text{ref}}=0.413 \text{ h}^{-1}$).

At the above conditions, increasing h_o to 6 returns the left simulation of Figure 8 and setting standard deviation (SD) of h_o equal to 2 (instead of the default 0) adds variability in lag time as shown on the right graph of Figure 10.

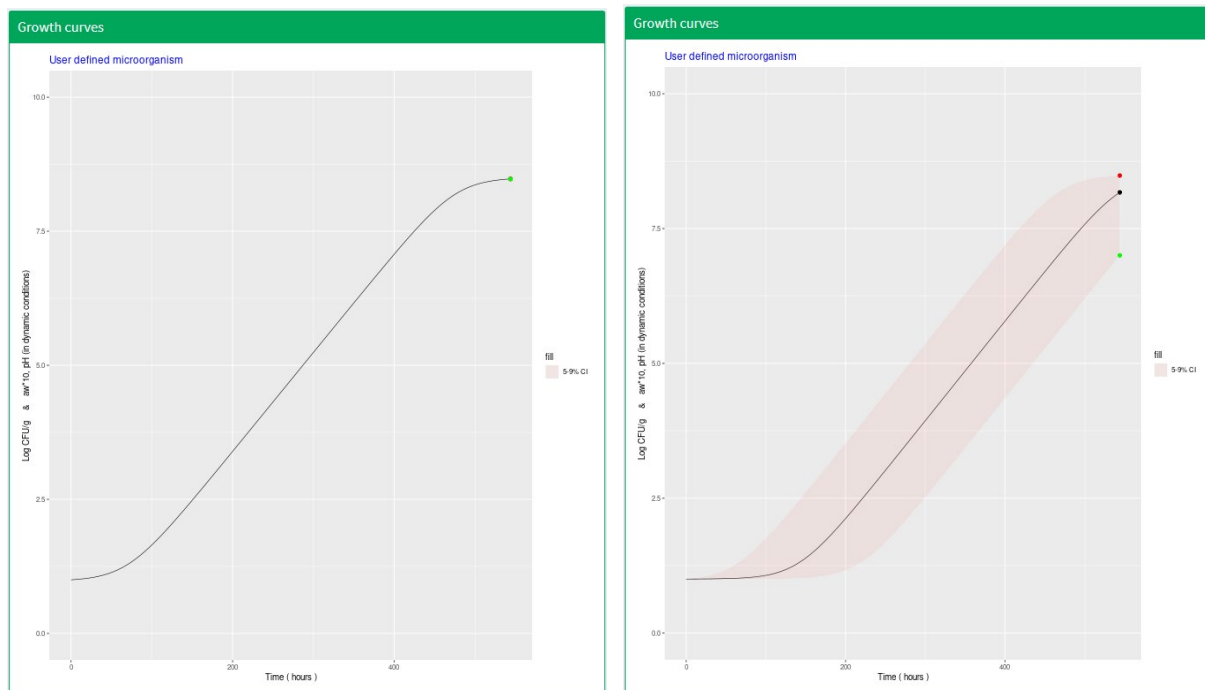


Figure 10. Graph with fixed lag time (left) and lag time derived from normal distribution of h_o with mean value 6 and SD 2 (right). The dots show the $\log N$ that correspond to the 5 (●), 50 (median; ●) and 95% percentile (●) of the normal distribution of h_o . The symbols have the same meaning in all graphical outputs of growth simulations, herein.

The changes in h_o and SD are typed at the bottom of the “Intr/Extrinsic factors” tab.

Work-To-Be-Done (h_o : 0.1 to infinity)

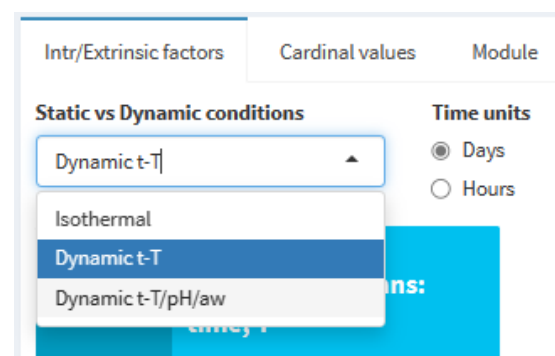
0.01

sd h_o (stochastic lag)

1

2.5 Outputs under dynamic conditions

Simulation under dynamic conditions can be carried out by selecting “Dynamic t-T” for dynamic T profile, or “Dynamic-t-T/pH/aw”, for dynamic T-pH-aw datasets.



Dynamic data set may be either uploaded with an XL file or manually typed/pasted in a table in the tool.

It is very important to define the proper “*Interpolation step*”, as follows:

- (a) The “*Interpolation time step*” is dependent on the T, pH and a_w steps of the imported dataset. Since it also impacts the resolution of simulation time, it is recommended not not to be higher than half of the minimum time step of the imported data set, again not necessarily of the first interval. Selecting the proper value should aim to maximize the dt intervals between two consecutive interpolated values of time and T, pH and a_w .

In the following typical example (i.e., from an XL file):

hours	T	pH	a_w
0	10	6.25	0.98
6	10	6.25	0.98
22	10	6.035	0.98
29	10	6.035	0.98
46	10	5.935	0.95
53	10	5.935	0.95
69	10	5.685	0.95
78	10	5.685	0.95
99	10	5.615	0.93
117	10	5.615	0.93
128	10	5.615	0.93
140	10	5.06	0.93
...

The minimum time step seems to be the first one, i.e., 6 h. Thus, a reasonable “*interpolation step*” could be between 0.6 and 1.2 (i.e., 10 or 5 times lower), but certainly not equal or higher than 6, which is the minimum recorded time step in the dynamic dataset uploaded.

TROUBLESHOOTING: If the “*interpolation time step*” is equal or higher than the minimum time step of the imported data set, i.e., 6 in the above example), the following error message will appear at the top of the graph:

“Error: An error has occurred. Check your logs or contact the app author for clarification.”.

The same error occurs in any of the following cases:

- “*Interpolation step*” are set to 0 (either on purpose, by mistyping, or instantly, while typing the decimal places).
NOTE: The simulation output is updated in real time during typing in the input boxes, or while moving the input sliders.

- *Work-to-be done* is typed as 0 (minimum acceptable value is 0.01)
- “*Maximum population density*” (N_{max}) is lower than the “*Initial contamination (Log CFU/g or ml)*”
- T_{ref} is lower than the T_{min}

Once the user selects the type of dynamic data set, then the proper box is prompting the user to upload a dynamic data set appears below (Figure 11) by locating the file to be uploaded on the PC (“Browse” button).

Figure 11. Uploading a dynamic dataset.

The graphical output window remains blank until the file is uploaded. Once it is uploaded the growth curve is updated by showing the Log N vs time along with the recorded values of T (*Dynamic time-T file*, Figure 12), or T , pH and a_w (*Dynamic time-T/pH/aw file*, Figure 13). The remaining functions, i.e., stochastic growth limits or adjusting the h_0 with or without SD remain as pre-selected. Furthermore, the user may still adjust the constant value of the other inhibitors 1 to 6, *via* the sliders, as explained above.

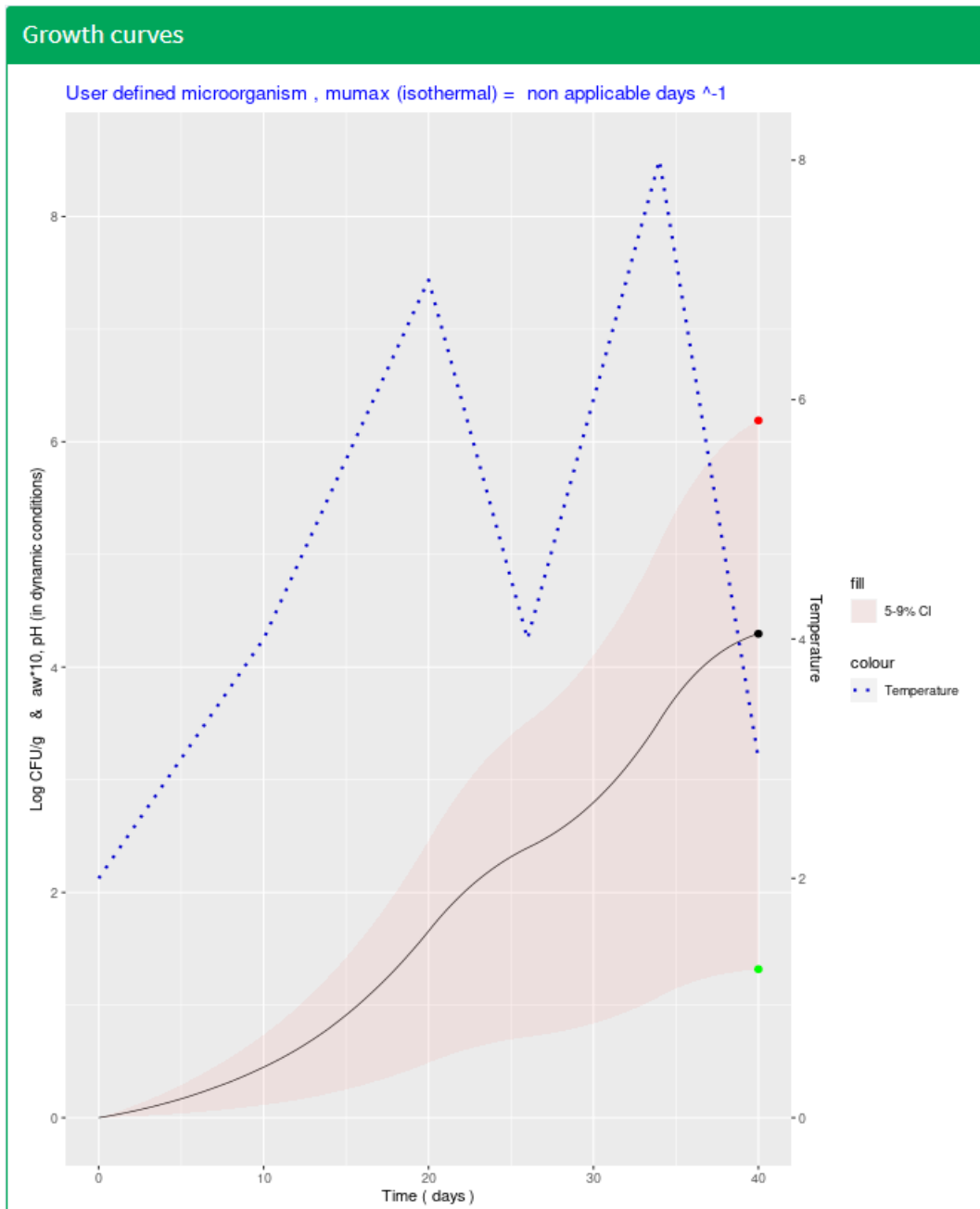


Figure 12. Growth simulation of the gamma model **without** interactions, under dynamic (non-isothermal) profile at pH 6.56, a_w 0.97, 25 ppm of inhibitor 1 (the rest set as 0) and μ_{ref} 0.413 h^{-1} at T_{ret} 25°C. The shaded area represents the prediction range, considering variability (200 iterations) in h_o and the cardinal values, herein only T_{min} by selecting “Normal dist.” from the drop-down list of “cardinal” tab. T_{min} follows a normal distribution with average -0.92°C and SD 0.5, and h_o follows a normal distribution with average 0.2 and SD 0.6. The rest model inputs maintain the default values of the tool. The limits of the shaded area represent the 5 (●), 50 (median; ●) and 95% percentile (●) of the resulting distribution of Log CFU/g at dt interval.

Interpolation step=0.5. **Example XL file: Time-T.xlsx.**

Growth curves

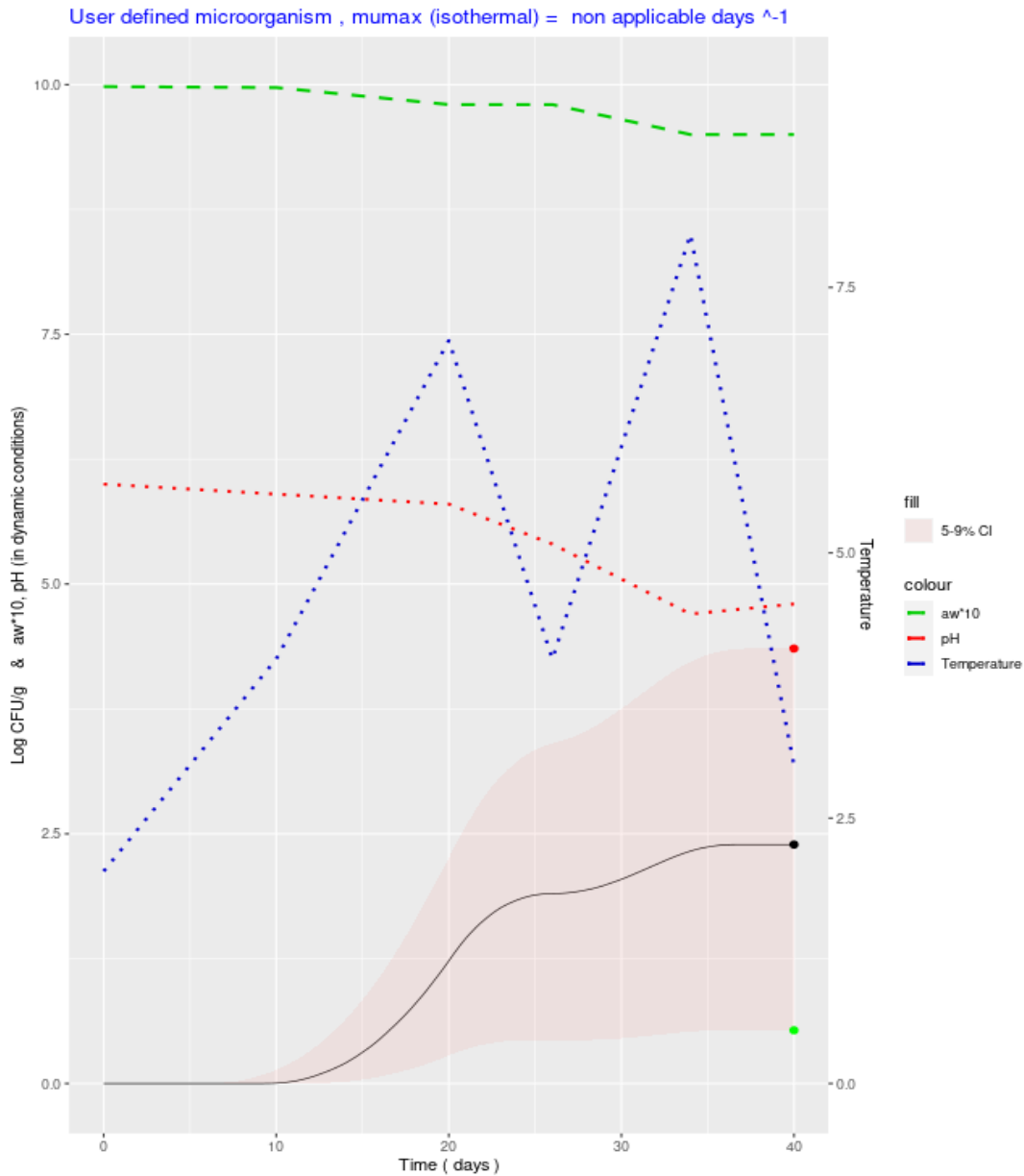


Figure 13. Growth simulation of the gamma model **with** interactions, under dynamic (non-isothermal) profile with default values of μ_{ref} 0.413 h⁻¹ at T_{ret} 25°C and no inhibitors (=0). The shaded area represents the prediction range, considering variability (200 iterations) in h_o and the cardinal values, herein only T_{min} by selecting “Normal dist.” from the drop-down list of “cardinal” tab. T_{min} follows a normal distribution with average -0.92°C and SD 0.5, and h_o follows a normal distribution with average 0.2 and SD 0.6. The limits of the shaded area represent the 5 (●), 50 (median; ●) and 95% percentile (●) of the resulting distribution of Log CFU/g at dt interval. Interpolation step=0.2. Example **XL file: Time-T-pH-aw.xlsx**.

2.6. Tab “Module”: appending a heating step after storage

This tab offers the option of applying a heating step at the end of growth simulation. The log reductions caused by heating are calculated based on the duration of the heating process (=heating time), the heating temperature, the D-value (D_{ref}) of the target organism at a reference temperature (T_{ref}) and the associated Z value, which describes the dependency of the D-value on temperature (Figure 14). The inclusion or exclusion of the heating step are controlled by interchangeably clicking the “Growth & Inactivation” and “Growth” radio buttons shown in the screenshot above.

Intr/Extrinsic factors
Cardinal values
Module

Select modules

☒ Growth

☐ Growth & Inactivation

Dref (min)

38

Tref

54

Z

7

Heating Temp

48
57
100

485460667278849096100

Heating time (min)

0
12
140

014284256708498112126140

The log reductions ($LogR$) are calculated by the Bigelow model converting the cooking time ($time$) at a specified temperature (T) as equivalent time at a reference temperature (T_{ref}). The following equations are used in the calculations:

$$LogR = \frac{time}{D_T} \quad (\text{Equation 14})$$

where D_T is the D-value at the cooking temperature T . It is estimated as a function of the D value at the T_{ref} , i.e., D_{ref} , and the Z value, i.e., the temperature change that causes a 10-fold change in the D-value, based on the following equation:

$$D_T = D_{ref} \cdot 10^{\frac{T_{ref}-T}{Z}} \quad (\text{Equation 15})$$

Figure 14. Panel of input values for the heating step at the “Module” tab.

The module is applicable to growth simulations under both static or dynamic conditions and the pre-selected options of deterministic, stochastic, or dbase-derived cardinal values (Figure 15), as described above, remain active.

Growth curves

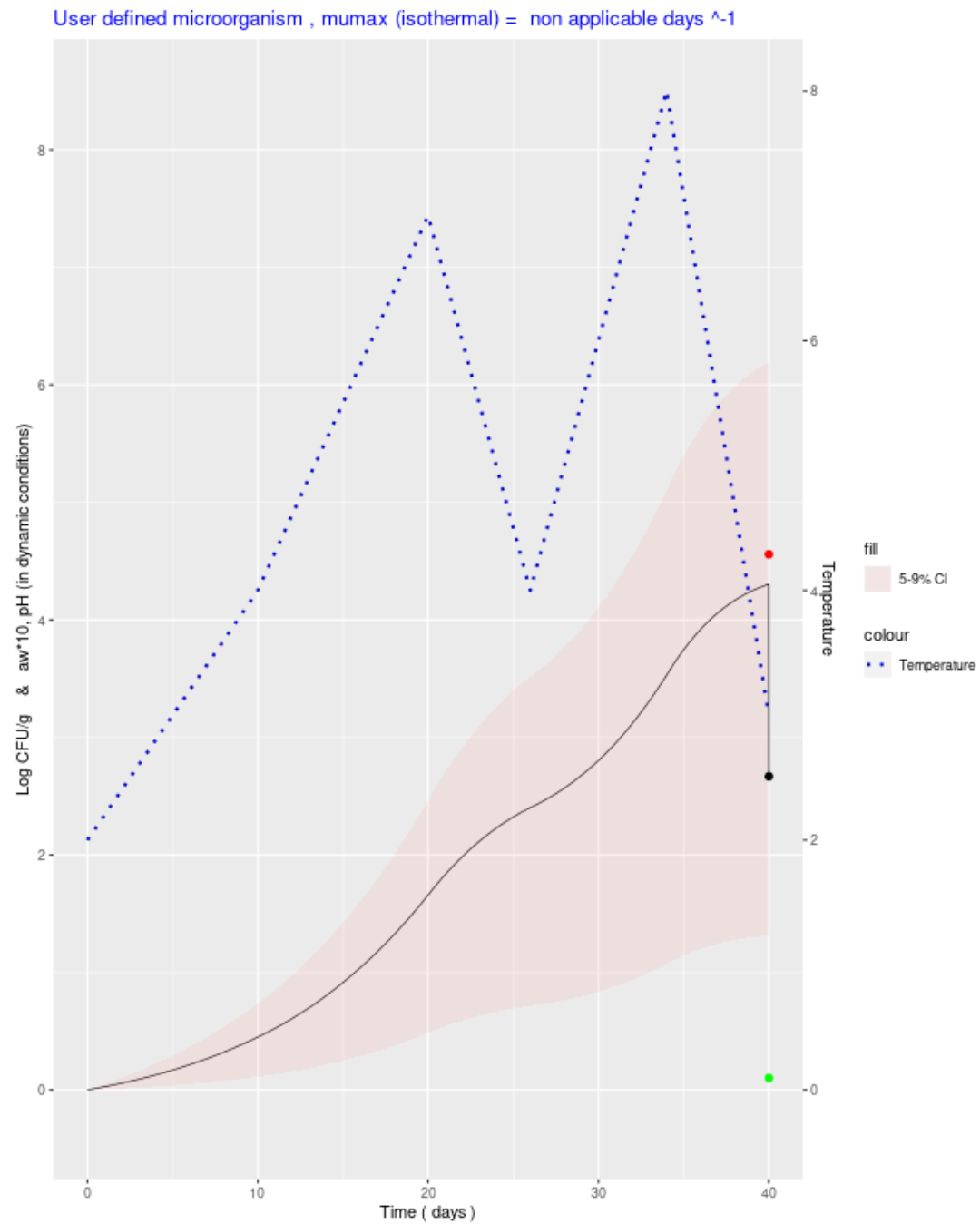


Figure 15. The dynamic growth simulation of Figure 12, followed by a heating step at 57°C for 12 minutes, with $D_{ref}=38$ min, $T_{ref}=54^{\circ}\text{C}$ and $Z = 7^{\circ}\text{C}$. The colored dots represent the downshifts of LogN due to heating, corresponding to the 5 (●), 50 (median; ●) and 95% percentile (●) of the prediction range by the end of storage, which encompasses the variability in T_{min} and h_0 .

2.6. Adding independent experimental data & downloading the model outputs

The user may overlay independent experimental data for comparison with model simulations (Figure 16). From the same menu, the data may be cleared, too. Once they are loaded, they remain in the background and show up again when switching to “Import data”.

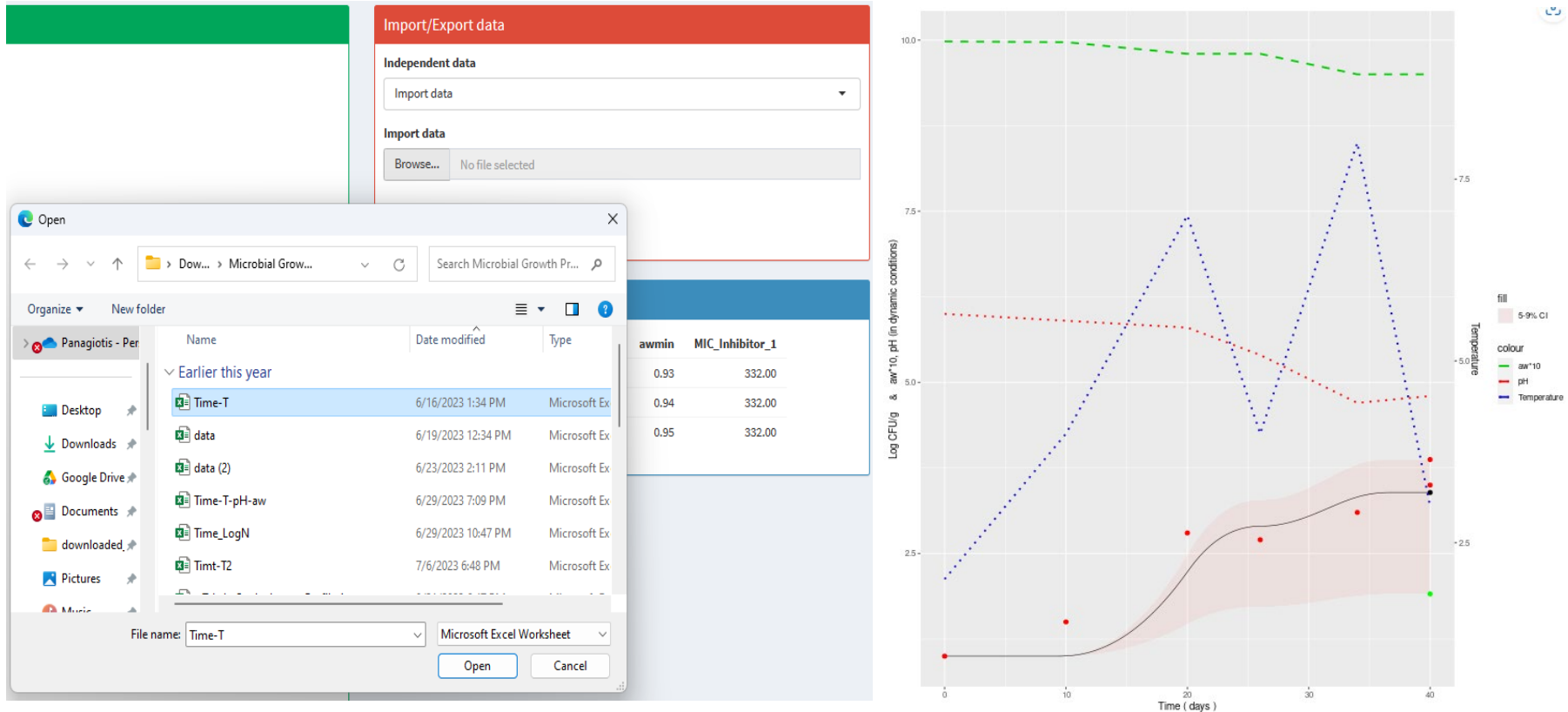


Figure 16. Importing independent experimental data (left snapshot) for comparison with model simulations (right snapshot) of Figure 13. Initial contamination 2 Log CFU/ml or g and $h_0=0.4$ with SD 0. Cardinal values are defined as “Normal dist.” with SD of T_{min} (-0.92°C) 0.3°C, SD of pH_{min} (4.4) 0.1 and of aw_{min} (0.915) 0.001. Observed data from example XL file: **Time_LogN.xlsx**

The output of model simulations can be extracted to an XL file, by clicking on the “Download Model Outputs”. The columns of the XL file are divided in the following categories, depending on whether simulations took place under static or dynamic conditions (Figure 17):

(a) *Isothermal conditions*

1. **A-F:** Model simulations, i.e., time (column **A**), mean predictions (column **B**), high 95% confidence interval (column **C**) and low 95% confidence interval (column **D**). Column **E** shows whether Rosso temperature gamma term is enabled (“TRUE”) or not (“FALSE”). Column **F** reports the predicted μ_{\max} .
2. **G-O:** Input values of all independent variables including the 6 inhibitors.
3. **P-AB:** Fixed cardinal values (deterministic approach). These are the values that appear under “Fixed value” in the “Cardinal values” tab. When Rosso gamma temperature term is enabled in “Cardinal values” tab, then T_{ref} represents T_{opt} and μ_{ref} represents μ_{opt} .
4. **AC-AG:** Average cardinal values following normal distribution. The *SD* values are not extracted, but remain in the tool and can be accessed when “Normal dist.” is selected in the “Cardinal values” tab.

(b) *Non-isothermal conditions (“Dynamic time- T ”), or dynamic T , pH and a_w conditions (“Dynamic time- $T/pH/a_w$ ”)*

1. **A-D:** Model simulations, i.e., time (column **A**), mean predictions (column **B**), high 95% confidence interval (column **C**) and low 95% confidence interval (column **D**). Column **E** shows whether Rosso temperature gamma term is enabled (“TRUE”) or not (“FALSE”). A single predicted μ_{\max} is not applicable in dynamic profiles.
2. **F-N:** Input values (including interpolated dynamic values of the appropriate variables).
3. **O-AA:** Fixed cardinal values (deterministic approach). These are the values that appear under “Fixed value” in the “Cardinal values” tab. When Rosso gamma temperature term is enabled in “Cardinal values” tab, then T_{ref} represents T_{opt} and μ_{ref} represents μ_{opt} .
4. **AB-AF:** Average cardinal values following normal distribution. The *SD* values are not stored. They appear when “Normal dist.” is selected in the “Cardinal values” tab.

The structure of the XL file is shown in the next page.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF			
1	Model simulations					Input values of independent variables (including interpolated dynamic values, if relevant)										Fixed (deterministic) cardinal values										Average cardinal values following normal distribution									
2	x	y	High35	Low05	Rosso	T	pH	aw	Nitrites	CO2	Phenolics	Lactate	Acetate	Sorbate	Cardinal_Lvi	Tref	muref	Tmin	Tmax	awmin	pHmin	MIC_Nitrites	MIC_CO2	MIC_Phenc	MIC_Lactat	MIC_Acetal	MIC_Sorbat	Tmin_Norm	Tmax_Norm	awmin_Norm	pHmin_Norm	MIC_Nitrites			
3		0	0	0	FALSE	4	6	0.998	25	0	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
4		0.2	0.012696	0.016388	0.003277	FALSE	4.06	5.998	0.99798	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
5		0.2	0.012696	0.016388	0.003277	FALSE	4.06	5.998	0.99798	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
6		0.4	0.025699	0.033116	0.018823	FALSE	4.12	5.996	0.99796	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
7		0.4	0.025699	0.033116	0.018823	FALSE	4.12	5.996	0.99796	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
8		0.6	0.039013	0.050185	0.028643	FALSE	4.18	5.994	0.99794	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
9		0.6	0.039013	0.050185	0.028643	FALSE	4.18	5.994	0.99794	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
10		0.8	0.05264	0.0676	0.03874	FALSE	4.24	5.992	0.99792	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
11		0.8	0.05264	0.0676	0.03874	FALSE	4.24	5.992	0.99792	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
12		1	0.066585	0.085365	0.049118	FALSE	4.3	5.99	0.9979	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
13		1	0.066585	0.085365	0.049118	FALSE	4.3	5.99	0.9979	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		
14		1.2	0.080852	0.103482	0.05978	FALSE	4.36	5.988	0.99788	25	0	0	0	0	0	Normal dist.	25	0.413	-0.92	48	0.915	4.4	332	80	20	50000	15000	2500	-0.92	48	0.915	4.4	332		

Figure 17. Representative structure of an XL file that accommodates the output of user-defined model under non-isothermal (“Dynamic time- T ”), or dynamic conditions of temperature, pH and a_w (“Dynamic time- T - pH - a_w ”). At isothermal conditions, an extra column (**F**) is introduced with the predicted μ_{\max} in the proper units (h^{-1} or $days^{-1}$), that is not applicable under dynamic conditions. The remaining columns are shifted by one column on the right.

NOTE: The XL file created by the system is colorless. The titles have been added for the needs of the user guide.

An additional output is the estimation of time needed for certain user-defined log reductions in the down-right box:

Estimating time for certain log increase

Log increase

3

From

To

Average

11.6 days

21.9 days

14.4 days

2.7. Growth/No growth interface

In most updated version of the tool, under the “*User define*” module, the graphical outputs in the middle of the screen, are split in two tabs, one showing all **growth simulations** (dynamically updated) that have been described so far, and one, where the **growth/no growth interface** is plotted (Figure 18).

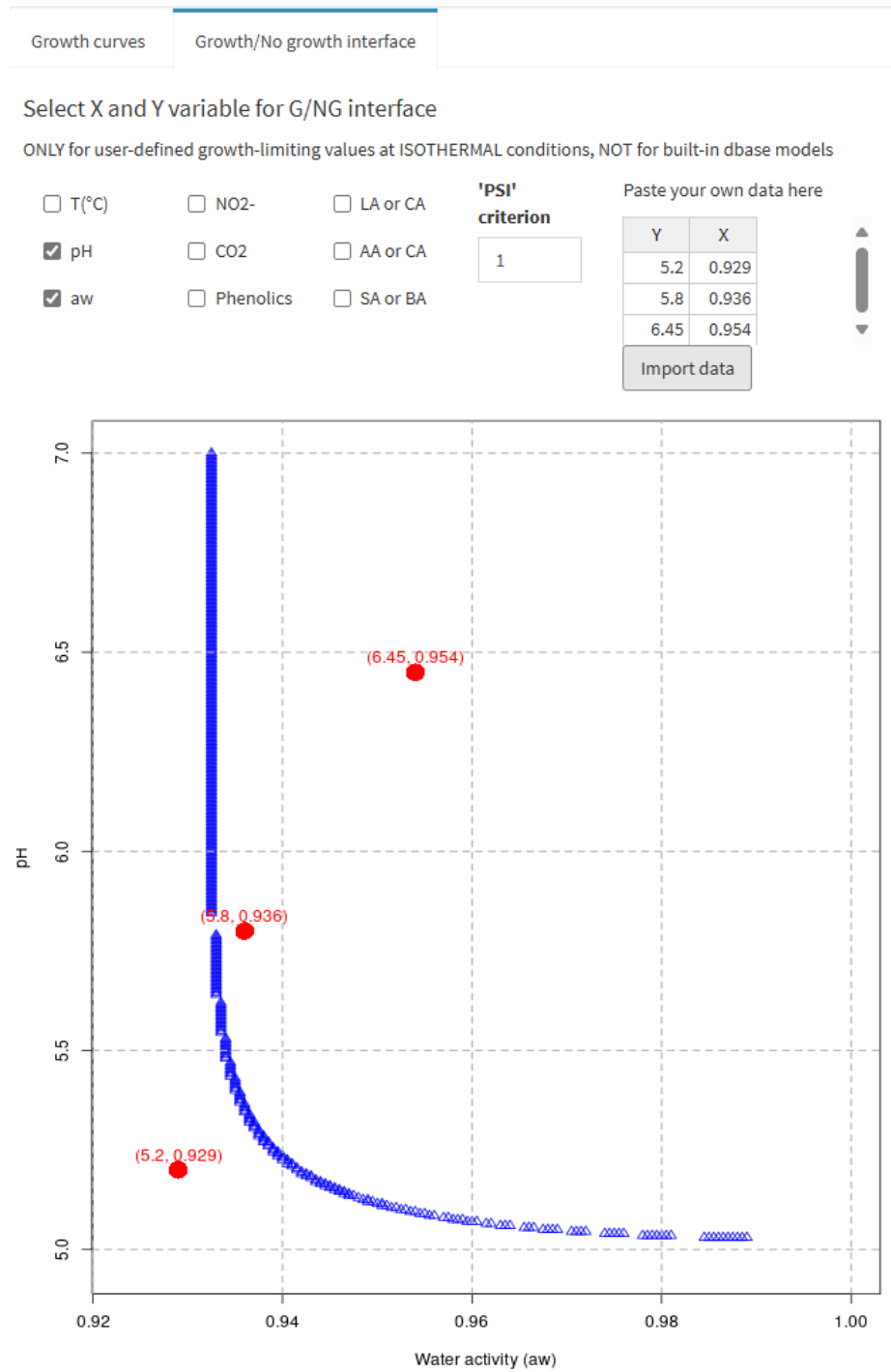


Figure 18. Growth/no growth interface in Growth Predictor.

The interface is generated based on the equations 3 to 8, by identifying all possible dual combinations of the X and Y variables selected that result in the same “ ψ ” value of 1 (default), or else (user-defined). The user may (and need to) select any possible combination of two factors (as Y and X) out of the 9 factors in total, presented in the checkboxes. Following selection, the growth interface of Figure 18 is automatically generated. For the rest of factors that are not plotted, their single value defined by the user on the lefthand panel of “user defined” are used. The interface is updated automatically in response to any change of the value of the non-plotted variables.

3. Module “Imported ‘e-Platon’ file”

This module is intended to predict pathogen growth in response to the pH, a_w and concentration of nitrites (ppm) of meat products as stored in the **e-Platon** (Microsoft Power BI) food safety database. To do so, the user needs to upload an XL file that contains the product names and their (aforementioned) characteristics, as well as the registered cardinal values of the biohazard of concern, e.g., *Listeria monocytogenes* (as default organism) in the following format (Figure 19):

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q					
1	SAMPLE T Sample Description		Sample Code	30100 pH	30110 Wat	20150 Salr	20190Liste	30160 Nitr	Mumax_R	61112 List	61122 List	61132 List	NO3_Min	T_Ref	LM pH	Ref	Uaw	Ref	U	NO3	Ref	LM
2	MEAT PRE KARAMANLIS SAUSAGES		18 283 027E	5.87	0.95	0.00	0.00	0.00	0.41	-0.50	4.43	0.92	353.21	25.00	5.70	0.99	0.00					
3	MEAT PRE TURKEY FRANKS		18 355 021E	6.45	0.96	0.00	0.00	6.80	0.42	-1.58	4.56	0.92	338.61	25.00	5.70	0.99	0.00					
4	MEAT PRE TURKEY FRANKS		19 268 016E	6.06	0.96	0.00	0.00	4.30	0.41	-0.75	4.37	0.93	342.64	25.00	5.70	0.99	0.00					
5	MEAT PRE TURKEY FRANKS		20 181 069E	6.22	0.97	0.00	0.00	14.80	0.41	-1.58	4.57	0.92	335.69	25.00	5.70	0.99	0.00					
6	MEAT PRE BREADED CHICKEN		20 134 008E	6.62	0.97	0.00	0.00	0.00	0.41	-1.45	4.34	0.92	351.59	25.00	5.70	0.99	0.00					
7	MEAT PRE KARAMANLIS SAUSAGES		18 341 049E	6.16	0.98	0.00	1.00	2.00	0.42	-1.47	4.48	0.92	327.99	25.00	5.70	0.99	0.00					
8	MEAT PRE TURKEY FRANKS		19 093 017E	6.13	0.98	0.00	0.00	8.00	0.42	-1.13	4.52	0.92	339.49	25.00	5.70	0.99	0.00					
9	MEAT PRE BREADED CHICKEN		20 134 008E	6.63	0.98	0.00	0.00	0.00	0.41	-1.45	4.34	0.92	351.59	25.00	5.70	0.99	0.00					
10																						
11	Applied filters: SAMPLE TYPE GROUPS is MEAT PREPARATIONS Sample Description is ΚΟΤΟΜΠΟΥΚΙΕΣ ΠΑΝΕ, ΛΟΥΚΑΝΙΚΑ ΚΑΡΑΜΑΝΛΙΔΙΚΑ, or ΛΟΥΚΑΝΙΚΑ ΠΟΥΛΕΡΙΚΩΝ T. ΦΡΑΝΚΦΟΥΡΤΗΣ																					

Figure 19. Representative format of **e-Platon** file compatible with the “Imported ‘e-Platon’ file” module of **Growth Predictor**.

The file is uploaded through the following button:

Intr/Extrinsic factors

Cardinal values

Module

Import data (XL) file

Browse...

No file selected

Once the file of the above format is uploaded, the tool generates in the same graph, multiple growth simulations for the pathogen of concern in all products, based on their individual characteristics, which appear tabularly on the right of the screen (Figure 20). The simulations refer to days of storage and are based on the same primary and secondary gamma models (equations 1-

Product selection

Select product

All products

7, 12-13) as in “*User-defined conditions*”, without the option to use any of the Rosso terms, neither the interaction term “ ξ ”. The user may adjust the storage time (days) and temperature of the simulation, without being able to alter the remaining intrinsic characteristics of the products, which are pre-defined in the database and uploaded *via* the XL file. Testing the impact of alternative values for pH , a_w , or levels of multiple inhibitors on microbial growth, is only applicable in the module “*User-defined conditions*”, as detailed in the previous session.

As long as the selected option in the box “*Product selection*” remains “*All products*”, the tabs “*Cardinal values*” and “*Module*” and their associated numeric or slider inputs remain inactive. They become active when the “*Pick up a product*” is selected on the right window. The cardinal values in “*All products*” mode derive from the imported XL file and cannot be changed by the user, who can refer to the XL file to read them. Once the values are changed in the XL, the file needs to be re-loaded to update the graphs.

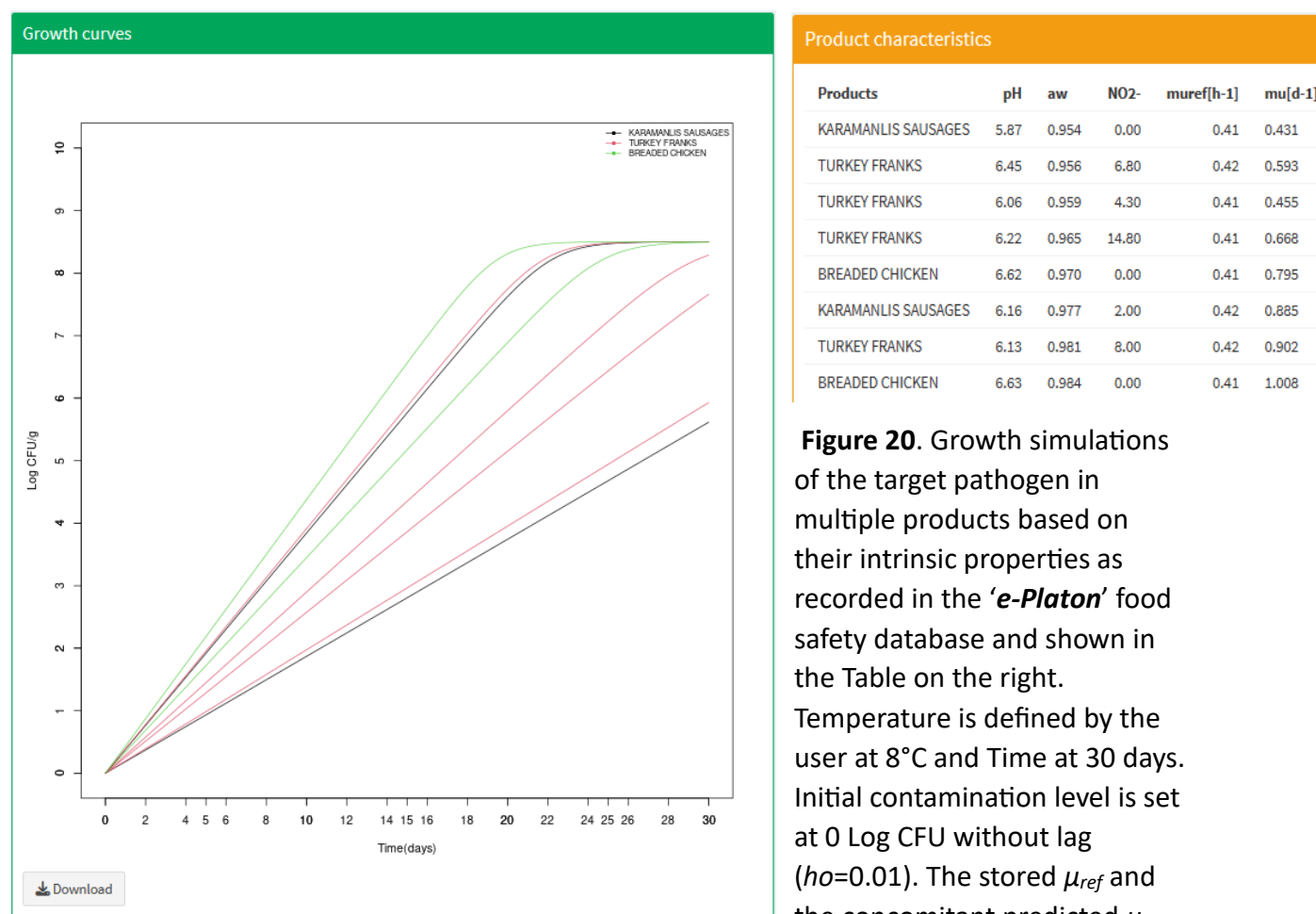


Figure 20. Growth simulations of the target pathogen in multiple products based on their intrinsic properties as recorded in the ‘*e-Platon*’ food safety database and shown in the Table on the right. Temperature is defined by the user at 8°C and Time at 30 days. Initial contamination level is set at 0 Log CFU without lag ($h_0=0.01$). The stored μ_{ref} and the concomitant predicted μ_{max}

values at the aforementioned time-temperature conditions of the formulated products are also shown at the last 2 columns of the right table and so are for when a single product is selected, or cardinal values are chosen from those available in the tool dbase. **Example XL file: data.xlsx.**

When “*Pick up a product*” is selected, the products are numbered from 1 to the maximum number different products extracted in the XL. The user selects the different product through their increasing numbering. This can be done by adjusting the product numbering by pressing the up and down arrows of the following box (Figure 21).

NOTE: The cardinal values for a single product are those appearing in the “*Cardinal values*” tab, which are editable and most likely differ from those imported with the XL. As such, the original cardinal values stored in the XL file (for “*All products*”) are temporarily ignored, i.e., while in this mode, and the growth simulations for a single product may differ from those of the same product when “*All products*” are selected. The XL-based cardinal values are considered again when “*All products*” is re-selected.

Product selection

Select product

Pick up a product
▼

Product

2
⬆ ⬇ ⬆

Product characteristics

Products	pH	aw	NO2-	muref[h-1]	mu[d-1]
TURKEY FRANKS	6.45	0.956	6.80	0.42	0.5383
TURKEY FRANKS	6.06	0.959	4.30	0.41	0.5790
TURKEY FRANKS	6.22	0.965	14.80	0.41	0.6204
TURKEY FRANKS	6.13	0.981	8.00	0.42	0.8517

Arrows for increasing or decreasing the product numbering. Each number corresponds to a single product, the name of which appears on the right table (here “*Turkey Franks*”).

Figure 21. Selecting a single product from the list of extracted products in the XL. Here the product number #2 is selected. The specific product information appears on the right as when all products are selected (Figure 19).

The user may access the growth simulations for different products by increasing or decreasing the (integer) coding number of the product, e.g., 1, 2, 3, or more. If the maximum number of products included in the XL file is exceeded, then the following error message appears:

“Error: An error has occurred. Check your logs or contact the app author for clarification”.

This suggests that the user needs to press the ‘down’ button in product numbering to restore an acceptable coding number for the uploaded products.

The remaining features and tabs of this module, e.g., cardinal values, ho, heating module (at the end of storage), etc., work exactly as in the module “User defined conditions”. The only differences are the interchange between ‘days’ and ‘hours’ when adjusting storage time, and the option to use the Rosso gamma term (equation 9) for temperature are not available in this module. Furthermore, dynamic simulations are available only dynamic temperature profiles.

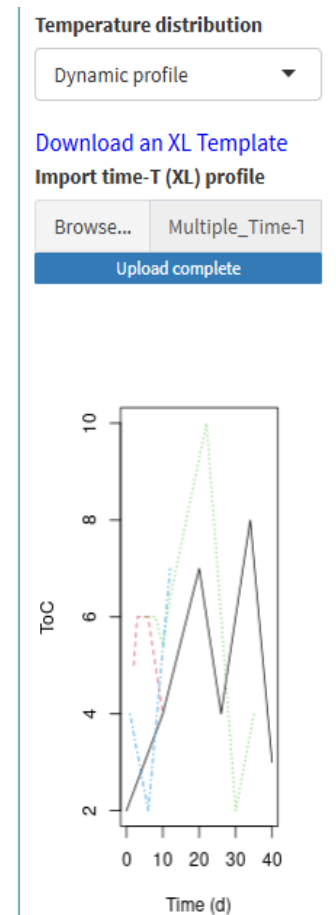
4. Module “Modular Process model”

The model is comprised of 4 modules, namely: (i) *Farm to end of processing*, (ii) *Processing to retail*, (iii) *Retail* and (iv) *Domestic storage*. The screen is split into two independently scrolling parts (Fig, 22). The left panel lists the model input variables from top to bottom and the right panel shows the model output distribution, i.e., the dose at the time of consumption or the logarithm of the probability of illness.

The input variables are the initial contamination or re-contamination (e.g., due to cross-contamination), the storage conditions (time and T), the product intrinsic properties (pH , a_w and one Inhibitor), the work-to-be-done (h_o) parameter associated with the lag time, the microorganism-specific cardinal values (including T_{ref} and μ_{ref}) and the Log reduction due to processing or cooking. The following variables can be introduced deterministically or stochastically, by selecting the type and parameters of specific distributions (normal, logistic, gamma, exponential, uniform or Pert), the list varying with the input variable as described in **Table 1**:

- Initial contamination (for the module “*From Farm to end of processing*”), or re-contamination (N_o), due to cross-contamination, at each of the vertical modules 2-4
- Storage time
- Storage temperature
- The product characteristics (pH , a_w and the level of 1 inhibitor)
- Final population density (N_{max})
- Log reduction or Log increase (if growth is not calculated via a growth model)
- The cardinal values as in the other two Modules, except for T_{ref} and μ_{ref} , which are introduced only deterministically. The Cardinal values can be introduced as fixed values, normal distribution or selected from the built-in dbase of the tool.
- The serving size

Furthermore, the time and temperature in modules 2 (distribution) and 3 (retails) may also be introduced as **multiple non-isothermal profiles** uploaded from an XL file. A link to a relevant template is provided when selecting “Dynamic profile” from the T drop down menu of each module as shown on the screenshot of this page (on the right). A preview of the uploaded profiles is provided (see the screenshot on the right).



The population of the hazard at each module is assumed to be randomly distributed in a serving size, thereby following a **Poisson** distribution ($\sim \text{Poisson}(N)$).

Growth in each of the 4 modules may be calculated based on the gamma growth model (Equation 1) with the interaction term “ ξ ” (Equations 2-7), in response to the above input variables or as a user-defined log increase (or log reduction) following a normal distribution with user-defined SD. The user may select or de-select the inclusion of the interaction term “ ξ ”, by the following checkbox: ☒ Growth model with interactions on the top right box.

In the output window, the user can set the number of iterations and choose among dose (Figure 22) and probability of illness (P_{ill} ; Figure 23) as model outputs, through the drop-down of “*Select plot*” box. The serving size is defined under the “*Exposure*” selection of the top list box, deterministically or stochastically. When selecting the “*Probability of illness*”, the user is prompted to define the prevalence (in “*Probability of illness*” selection of the top list box) and select one of the built-in dose-response models or a user-defined Exponential, Binomial and Beta Binomial dose-response model (Table 2). The built-in models include two for *L. monocytogenes* (susceptible and non-susceptible individuals), one for *Salmonella* and one for STEC. The latter considers both the variability of consumers susceptibility to the hazard, expressed as probability of illness by a single cell, and the associated uncertainty, expressed as probability distribution of the parameters describing the variability of consumer susceptibility. This entails a significantly higher computation time to deliver the probability of illness when simulating this model. When selecting a user-defined dose-response model, the user needs also to introduce the relevant parameters of the model. The list of built-in dose-response models is regularly expanded. For more specific models, please contact the administrator (pskan@aua.gr).

Table 1. Probability distributions used in “Growth Predictor” for the input variables.

<i>Variable</i>	<i>Available (variability) distributions</i>	<i>Parameters</i>
<i>Initial population or recontamination (N_o)</i>	Normal	Mean SD
	Pert	Min Mode Max
	Discrete	Manual typing/pasting to the <i>X</i> vector (No) vs <i>P</i> vector (Prob. of occurrence)
<i>Temperature (°C)</i>	Normal	Mean SD

	Logistic	Alpha Beta
	Gamma	Alpha Beta
	Uniform	Min Max
	Pert	Min Mode Max
	Discrete	Manual typing/pasting to the <i>X</i> vector (No) vs <i>P</i> vector (<i>Prob.</i> of occurrence)
	Dynamic profile	Uploaded from XL file with two columns (time/T). Available only for modules 2 and 3
<i>Product characteristics (pH, a_w, level of 1 inhibitor)</i>	Normal	Mean SD
<i>Time (days)</i>	Normal	Mean SD
	Exponential	Lamda Max time
	Logistic	Alpha Beta
	Gamma	Alpha Beta
	Triangular	Min Middle Max
	Pert	Min Mode Max

<i>Maximum population density (N_{max})</i>	Normal	Mean SD
	Uniform	Min Max
<i>Reduction (e.g., due to cooking or nonthermal inactivation)</i>	Normal	Mean SD
	Pert	Min Mode Max
	Uniform	Min Max
	Estimated by a thermal inactivation model with <i>Dref</i> , <i>Z</i> values as fixed values & time, <i>T</i> as fixed values or normal distributions	Mean time SD time Mean <i>T</i> SD <i>T</i>
	<i>Chardon and Evers, 2017</i>	
<i>Cardinal values of microbial growth: T, pH, a_w, MIC of inhibitor</i>	Normal	Mean SD
<i>Serving size (g)</i>	Normal	Mean SD
	Gamma	Alpha Beta

When the product properties, the values of *h₀*, or the cardinal values are changed, as well as when switching output type, the button below needs to be clicked to update the output graph.

[Click to render Plot or resample](#)

Clicking this button when no changes are made in any of the input variables, simply re-generates the output graph by resampling from the original input distributions, i.e., as a new simulation.

Apart from the output distributions (Figs. 22-23), the tool displays a Table with Exposure and illness metrics. These include the average and 5, 50 and 95% percentiles of the dose at the time of consumption and the logarithm of the probability of illness. The predicted annual cases depend on the defined “*Annual number of services*” (Fig, 24).

Table 2. List of dose-response models available in the tool.

Model	Microorganism	Formula	Parameters	Reference
Exponential	<i>L. monocytogenes</i> , susceptible population (elder)	$P = 1 - e^{-r \times Dose}$	r=8.39e-12	FAO and WHO, 2004; FDA, 2004 Chen et al. 2013
	<i>L. monocytogenes</i> , non-susceptible population		r=5.34e-14	
	Also available as user-defined model		r: user-defined	This tool
Beta-Poisson <i>It refers to the "Exact Beta-Poisson", i.e., a fit-for-purpose approximation of the Beta-Poisson</i>	<i>Salmonella</i> in eggs and broiler meat	$P = 1 - \left(1 + \frac{SF \times Dose}{\beta}\right)^{-\alpha}$	SF: scaling factor (1 by default) $B \sim \text{Triangular}(38.49, 57.69, 51.45)$ $\alpha \sim \text{Triangular}(0.0763, 0.2274, 0.1324)$	FAO/WHO, 2002
	Also available as user-defined model	$P = 1 - \left(1 + \frac{Dose}{\beta}\right)^{-\alpha}$	α, β : user-defined	This tool (Strachan et al. 2005)
Beta- Binomial	STEC in ground beef	$P = 1 - (1 - P_i(1))^{Dose}$	$P_i(1) \sim \text{Beta}(\alpha, \beta)$ $\alpha=0.267$ $\ln \beta \sim N(5.435, 2.47)$	Cassin et al. 1998
	Also available as user-defined model		α, β : user-defined, as parameters of Beta distribution	This tool
Binomial	Available only as user-defined model	$P = 1 - (1 - P_i(1))^{Dose}$	$P_i(1)=r$: user-defined	This tool

4.1. Partitioning, mixing and cross-contamination

The module enables the assessment of the impact of partitioning (fractionation), mixing and increase in prevalence and microbial load due to cross-contamination at the “*end of processing to retail*”, “*Retail*” (e.g., due to slicing or mixing) and “*Domestic environment*” (e.g., due to food preparation) on the final estimation of ingested dose and the probability of illness per serving, as well as the total number of cases per annum. The estimation formulas (acc. to the screenshot on the right) are as follows:

The screenshot shows a software interface with three sections: 'Partitioning', 'Initial mass (g: 0-infinity)', and 'Added mass (g: 0-infinity)'. The 'Partitioning' section has three radio buttons: 'No partition' (selected), 'Partition', and 'Mixing'. Below it, there are two input fields: 'Initial mass (g: 0-infinity)' with the value '1' and 'Added mass (g: 0-infinity)' with the value '1'. At the bottom, there is a section 'P cross-contamination (0-1)' with an input field containing the value '0'.

1. **Partitioning:** By selecting “*Partitioning*” (via the radio button) the user needs to define the partition coefficient (PP) by assigning a value from >0 (almost elimination of the batch) to 1 (no partitioning). For instance, a value of 0.5 suggests that the batch is divided in half. This apparently affects the distribution of microbial contamination in the sub-products resulting from the partitioning. The user is requested to also introduce the mass ($mass$) of the product that is partitioned. As such, if the product carries N_0 CFU/g prior to partitioning, then the population in the sub-products generated by partition follows a binomial distribution: $Binom(N_0 * mass, PP)$. The same concept applies to all consecutive modules, where partition is applied.
2. **Mixing:** Clicking on “*Mixing*” allows the user to define the **initial mass** (in g: 0 to infinity), i.e., the mass of the incoming product at a particular stage and the **added mass**, i.e., the mass of the product mixed with the incoming batch. A value of 0 for any of the two variables means that no product is added in the mixture, associated with the relevant mass part.
3. **P cross contamination:** This is a continuous value between 0 and 1 that informs the algorithm about the increase in the prevalence at that stage (starting from a given initial prevalence), due to cross-contamination at the current stage. In particular, if the previous prevalence is P , then a cross contamination probability P_{cc} will lead to a new prevalence (P_{new}), increased by the following amount: $P_{new} = P + (1 - P) \cdot P_{cc}$.
4. **Updating the concentration of the hazard at each stage considering mixing and cross-contamination:** When mixing an incoming product of $mass A$ carrying a microbial contamination of $\text{Log CFU}_A/\text{g}$, with new product mass of $mass B$ that introduces an additional contamination $\text{Log CFU}_B/\text{g}$, the microbial concentration of the resulting mixture ($\text{Log CFU}_{mix}/\text{g}$) is estimated as follows:

$$\text{Log} \frac{\text{CFU}_{\text{mix}}}{g} = \text{Log} \left(\frac{\text{CFU}_A/g}{\frac{\text{mass}_A + \text{mass}_B}{\text{mass}_A}} + \frac{\text{CFU}_B/g}{\frac{\text{mass}_A + \text{mass}_B}{\text{mass}_B}} \right) \quad (\text{equation 16})$$

taking into the dilution of the microbial concentration of the incoming product (mass A) due to mixing with the additional product mass B. Accordingly, the microbial concentration in the new product mass (*mass B*) is also diluted by mixing with the incoming batch and the concentration of the hazard coming from mass B, in the mixture becomes:

$$\text{Log} \frac{\text{CFU}_B}{g} = \text{Log} \frac{\text{CFU}_B/g}{\frac{\text{mass}_A + \text{mass}_B}{\text{mass}_B}} \quad (\text{equation 17})$$

To account both for cases where the incoming product is free of microbial contamination ($\text{Log} \frac{\text{CFU}_A}{g} = 0$), or carries a microbial load $\text{Log} \frac{\text{CFU}_A}{g} \neq 0$, the concentration of the microbial hazard in the mixture is assumed to follow a *Uniform* distribution:

$$\text{Log} \frac{\text{CFU}_{\text{mix}}}{g} \sim \text{Log} \{ \text{Uniform} [\min (\frac{\text{CFU}_A}{g}, \frac{\text{CFU}_B}{g}) , \max (\frac{\text{CFU}_{\text{mix}}}{g}, \frac{\text{CFU}_B}{g})] \} \quad (\text{equation 18})$$

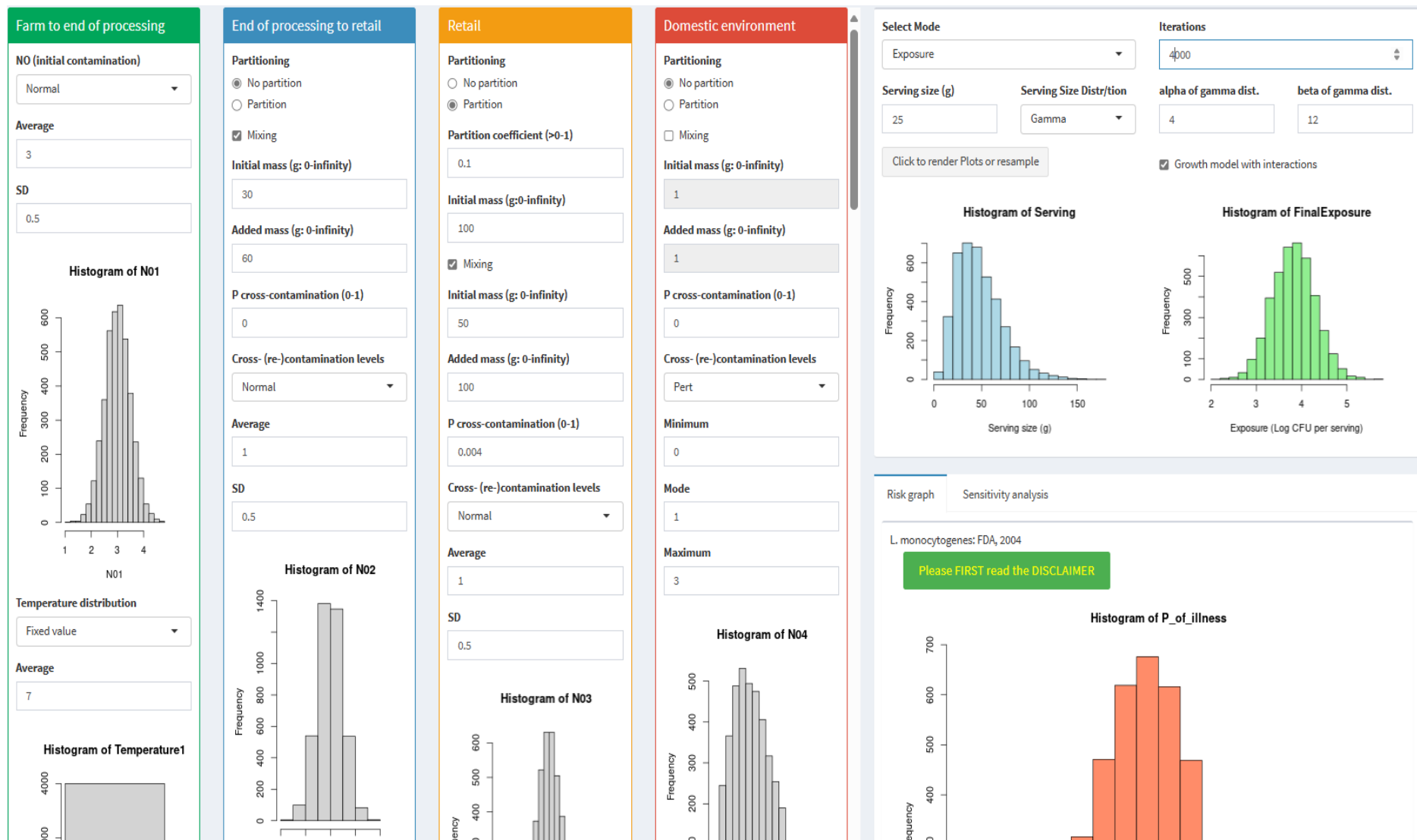


Figure 22. Generic output of the “Modular process model” module that shows the two independent scrolling windows for the input and output distribution (here, dose at the time of consumption), respectively. Remember to always press [Click to render Plot or resample](#) to update the output distribution, e.g., after changing any of the inputs or the number of iterations.

By selecting the “Probability of illness” in the “Select plot” box the user is asked to define the prevalence (0 to 1 = 0 to 100%) and select among different dose-response models from the drop-down list. Then by clicking the button “click to render Plots or resample” the orange distribution of Probability of illness shows up, along with the relevant numeric metrics on the bottom right of the right window (Figure 24).

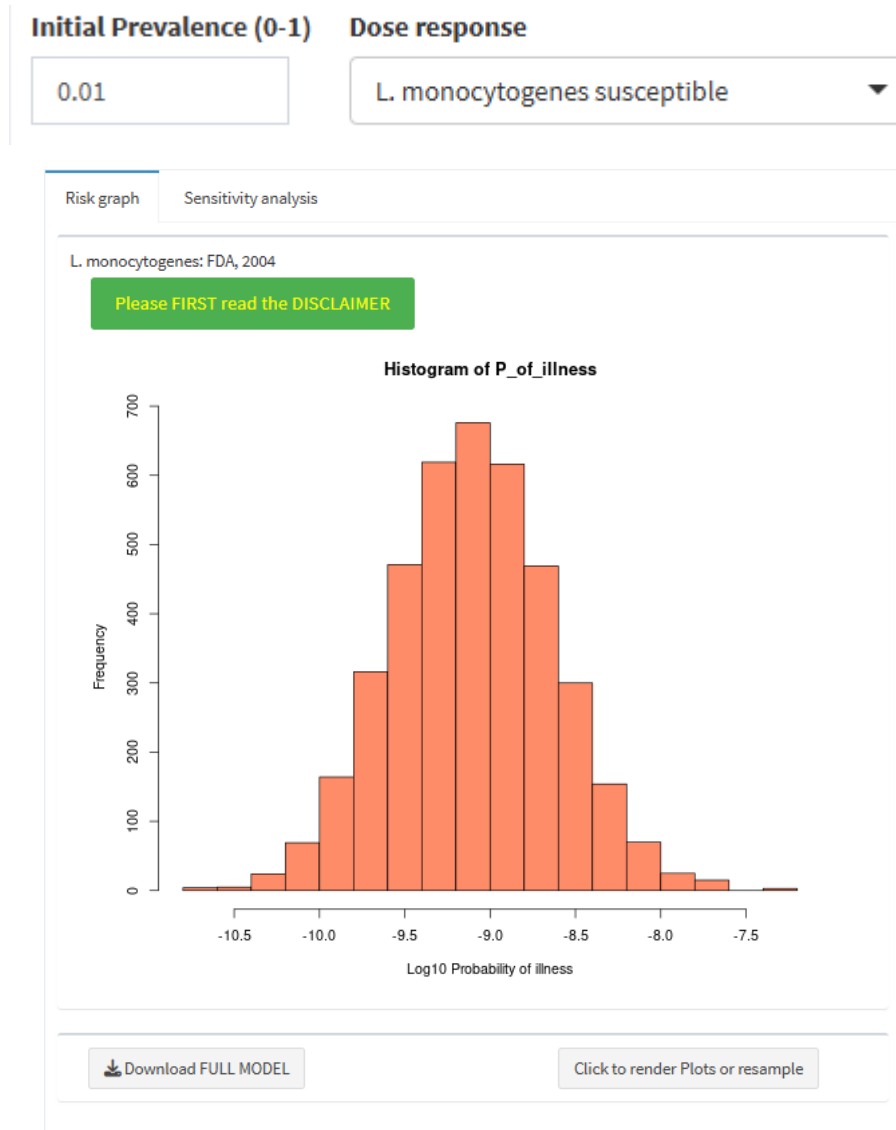


Figure 23. The QMRA output about the Probability of illness that appears on the right window of the screen. *Please refer to Figure 21 in the previous page for the actual right-hand side output of the tool. This is an indicative representation of variability distribution of P_{ill} , for clarity purposes.*

Exposure & Illness metrics			
Annual number of services			
1			
Exposure_Metrics	LogCFU_per_g_OR_ml	Illness_Metrics	Estimate
Average Dose per serving	5.7	Average Pill per serving	6.036e-07
-	-	Cases per annum	6.036e-07
Mode	5.5	Mode	3.162e-07
p5	4.6	p5	4.972e-08
p50	5.62	p50	5.233e-07
p95	6.89	p95	9.611e-06

Figure 24. Table of exposure and probability of illness metrics.

4.2 Sensitivity analysis

On the top of the box where the histogram of the probability of illness *per serving* is displayed, there appear two tabs, namely “*Risk graph*” and “*Sensitivity analysis*”. The “*Risk graph*” takes you to the histogram of \log_{10} Pill (per serving) as above (Fig. 23). The “*Sensitivity analysis*” tab displays a tornado graph (Figure 25) for the distribution of **exposure** or **Probability of illness per serving**, based on **Spearman** or **Pearson** correlation coefficients. Both the type of correlation coefficients and the target dependent variable of the sensitivity analysis, i.e., exposing dose or probability of illness *per serving* are user-defined. The tornado graph is 2-colored, one for variables with positive correlation and a separate one for the negative correlating variables. The list of variables included in the sensitivity analysis includes the following:

- Initial contamination
- Cross-contamination in modules 2 to 4.
- Log increase in modules 1 to 4: this practically reflects the log increase regardless of the initial level or the level of cross-contamination.
- Final level of modules 1 to 4: this is used to account for the variability in the levels of each module carried over to the next one without growth.
- Decontamination (for module 1)
- Cooking (for module 4)
- Total prevalence
- Serving size

Enabling a tornado graph requires at least 2 of the above variables having variability (i.e., stochastic inputs). If all inputs are deterministic, or not enough variability is provided for the variables involved in the analysis, a warning (error) message appears in the top right of the sensitivity analysis tab.

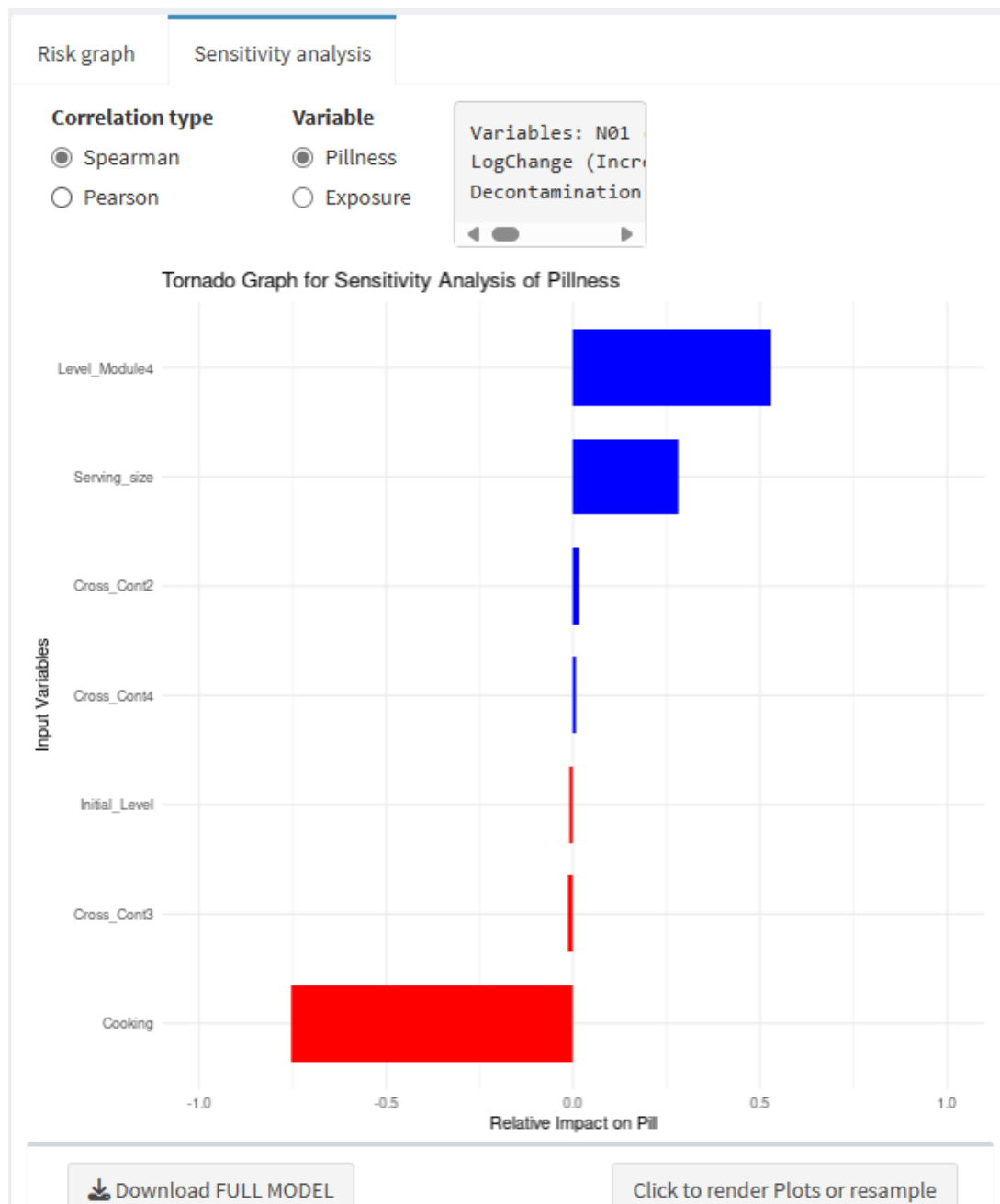


Figure 25. Tornado graph for the correlation coefficients used in the sensitivity analysis, for testing the relative impact of each of the above listed input variables on the QMRA output.

4.3. Uncertainty in prevalence

The uncertainty in the initial prevalence on the final prevalence (module 4) and the QMRA output, can be modelled with a beta distribution of the form:

Beta ($s+1, N-s+1$), where “ s ” is the number of positive samples and “ N ” is the total number of samples surveyed. The user may select to include or exclude the uncertainty of the initial prevalence by ticking and unticking the relevant checkbox, respectively:

<input checked="" type="checkbox"/> Beta dist. for uncertainty in prevalence	Positives	Total
	<input type="text" value="10"/>	<input type="text" value="100"/>

All relevant information about model inputs and outputs can be extracted to an XL file that contains 4 sheets:

1. “*Parameters*”: Overall model description that lists all the variables, specifying the user-inputs per variable.
2. “*Input distributions*”: the result of iteration for those stochastic model variables.
3. “*Dose*”: the iterations of the dose calculated by the simulation of the exposure model.
4. “*Pillness*”: the iterations of the Log P_{ill} calculated by the simulation of the exposure model and the selected dose response model.

NOTE: *The model practically runs with unlimited number of iterations. However, due to server limitations and for optimal speed, when more than 10.000 iterations are chosen the output is calculated for maximum 1500 iterations, whereas the input distributions are calculated based on the actual number of iterations. By resampling from the input distributions (with the relevant button), multiple simulations are performed that help to achieve a reliable output approximation.*

5. Module “*Estimation of cardinal values*”

This module offers the capacity to fit the gamma model of equation 1 to multivariate datasets uploaded by the user, for estimating the cardinal growth values of a microorganism. To improve the fit for the effect of the pH on μ_{max} , the gamma term for pH in equation 1 (termed “*pHmin-Presser*”) can be replaced by the Rosso “*cardinal*” model for pH (equation 19 below, derived from equation 9, but for $n=1$), or the relevant gamma term proposed by Aryani et al. (2015) (equation 10), which contains the parameter $pH_{1/2}$:

$$CM_1(pH) = \begin{cases} 0, & pH \leq pH_{min} \\ \frac{(pH - pH_{max})(pH - pH_{min})}{(pH_{opt} - pH_{min})(pH - pH_{opt}) - (pH_{opt} - pH_{max})(pH_{min} - pH)}, & pH_{min} < pH < pH_{max} \\ 0, & pH \geq pH_{max} \end{cases}$$

Equation (19)

The selection of either term can be made *via* a drop-down menu (“*pH gamma term*”) located at the top of the middle box of the screen (Figure 24). The option (“*pHmin-Presser*”) refers to the default gamma term for pH in equation 1. Similarly, there are 2 options available for fitting the temperature gamma term, namely, the original term in equation 1 (“*Tmin/Tref*”) and the Rosso “*cardinal*” term of equation 9. As for the pH gamma term, any of the two terms can be selected through the relevant “*T gamma term*” drop-down menu (Figure 26).

The effect of a_w on μ_{max} can also be described by fitting either the default (linear) a_w gamma term of equation 1, (termed “*awmin-Linear*”), or the cardinal model for a_w (equation 20), which derives from equation 9, by setting ‘ n ’ equal to 2, i.e., as for the temperature cardinal term:

$$CM_2(a_w) = \begin{cases} 0, & a_w \leq a_{wmin} \\ \frac{(a_w - a_{wmax})(a_w - a_{wmin})^2}{(a_{wopt} - a_{wmin})\{(a_{wopt} - a_{wmin})(a_w - a_{wopt}) - (a_{wopt} - a_{wmax})[a_{wopt} + a_{wmin} - a_w]\}}, & a_{wmin} < a_w < a_{wmax} \\ 0, & a_w \geq a_{wmax} \end{cases}$$

Equation (20)

Similarly to the alternative terms for pH and T , the appropriate term for a_w can be selected by the drop-down menu (“*aw gamma term*”). For details about the model parameters (e.g., cardinal values and exponents) please refer to page 6 of the guide. The process takes place *via* the following steps as shown in Figure 24:

1. The dataset to be fitted are uploaded in the form of an **XL file** or **manually typed/pasted in a table** provided by the tool. In case of XL file, the fitted data must be pasted in columns according

to the configuration of a template downloadable through the link provided (“[Click here to download an XL Template](#)”). The dependent variable is μ_{max} without transformation, or with square root or ln transformation, selected by the user after the fitting data are uploaded (Fig. 26). The independent variables can be either a single variable or any combination of T , pH , a_w and up to 3 *inhibitors*.

2. The independent variable to be plotted against μ_{max} is chosen by the drop-down menu, located next to the file upload button. This is imperative for the user to inspect the general trend of μ_{max} in response to the explanatory variables and adjust the starting values of cardinal parameters accordingly, prior to fitting.
3. Once the dataset is uploaded, the middle box gets populated with the list of numeric input boxes for the cardinal parameters associated only with the uploaded variables that contain data. The remaining variables are hidden. The boxes contain the default system values. The user may alter any of the starting values as well as (optionally) fix those, which need to be excluded from fitting. This is a key step to ensure convergence.
4. Clicking the “**Perform Fit**” button triggers non-linear regression of the model to the uploaded data, using “*Gausse-Newton*” algorithm. It is strongly advised that every time the user modifies or fixes one or more parameter starting values, the final list of starting values should be temporarily saved in memory by pressing “**Save values**”, before performing the fitting. The saved values can be restored after fitting, by pressing “**Restore values**” and maintained while the user navigates through the plots of the other variables, or used as reference for further adjustments, until satisfactory convergence occurs.
5. When convergence is reached, the remaining 3 boxes on the right become populated with the parameter estimates, the goodness-of-fit criteria and the predicted vs observed μ_{max} plot, respectively (Figure 27).

In parallel to displaying the fitted model outputs, the central graph (on the left) is updated with the predicted data points and 4 simulation lines of μ_{max} in response to the whole range of the pre-selected independent variable. Each continuous line corresponds to a fixed value of the concomitant non-displayed variables, at the 25, 50 and 75 and 100% intervals of their range used for model fitting (Figure 28).

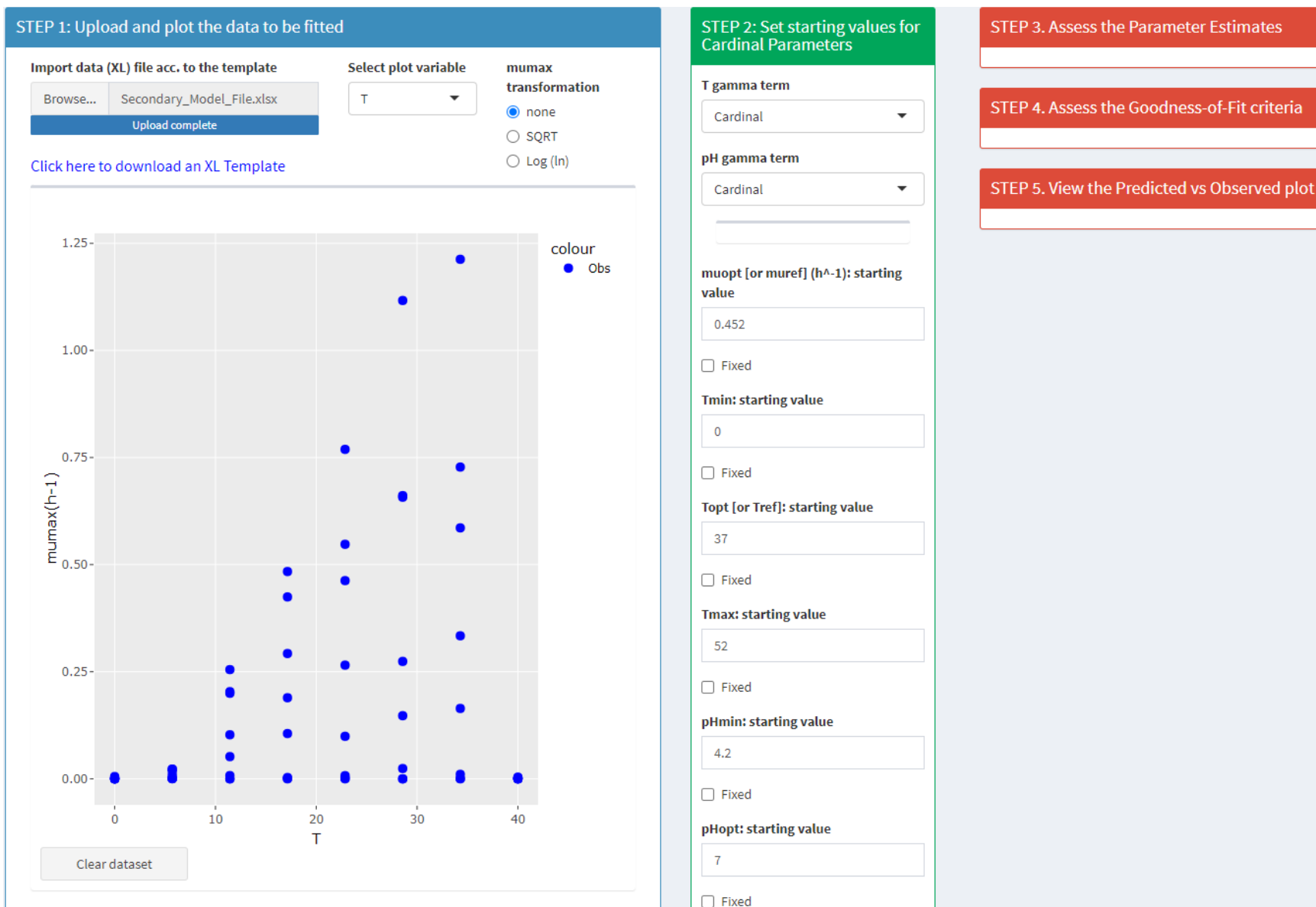


Figure 26. Generic output of the secondary fitting module referring to T as (single) independent variable.

STEP 3. Assess the Parameter Estimates

Parameter	Estimate	Std_Error	Percent_Rel_Std_Error
muopt	0.878	0.04691	5.343
Tmin	-1.591	3.24491	-203.949
Topt	32.161	1.09184	3.395
Tmax	40.008	0.16444	0.411
pHmin	5.197	0.04722	0.908
pHopt	6.572	0.06269	0.954
pHmax	6.999	0.00743	0.106

STEP 4. Assess the Goodness-of-Fit criteria

RMSE	R2	AIC	BIC
0.0984	0.873	-99.141	-81.87

STEP 5. View the Predicted vs Observed plot

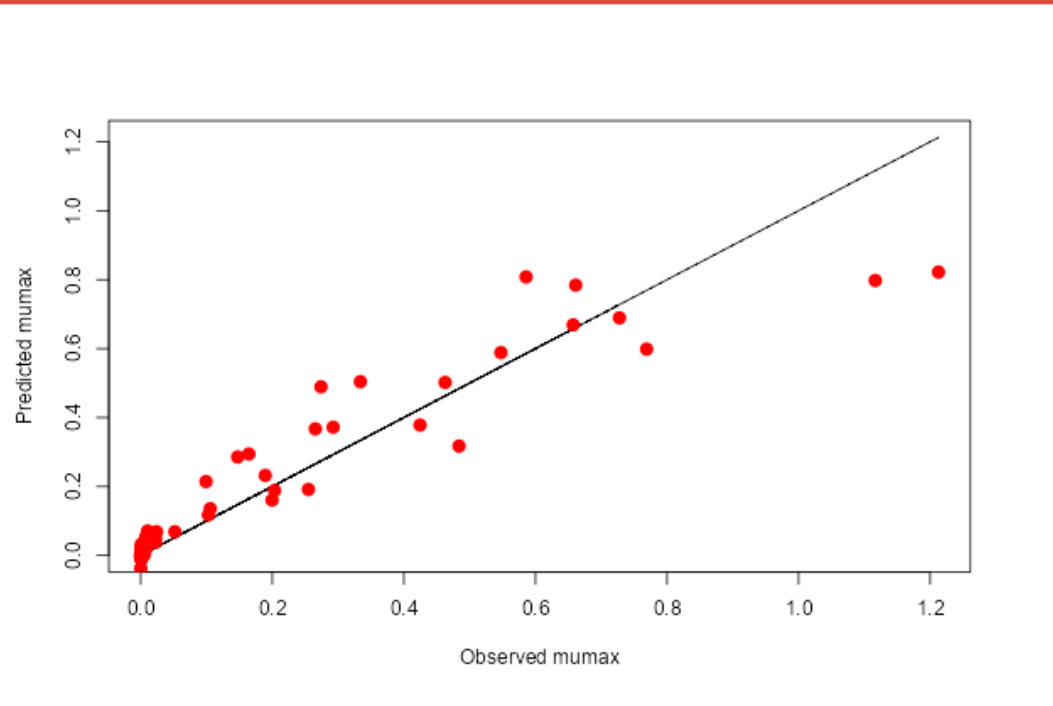


Figure 27. Fitted model outputs for T and pH as (the only) independent variables.

STEP 1: Upload and plot the data to be fitted

Import data (XL) file acc. to the template

Browse...

Secondary_Model_File.xlsx

Upload complete

Select variable to plot

T

[Click here to download an XL Template](#)

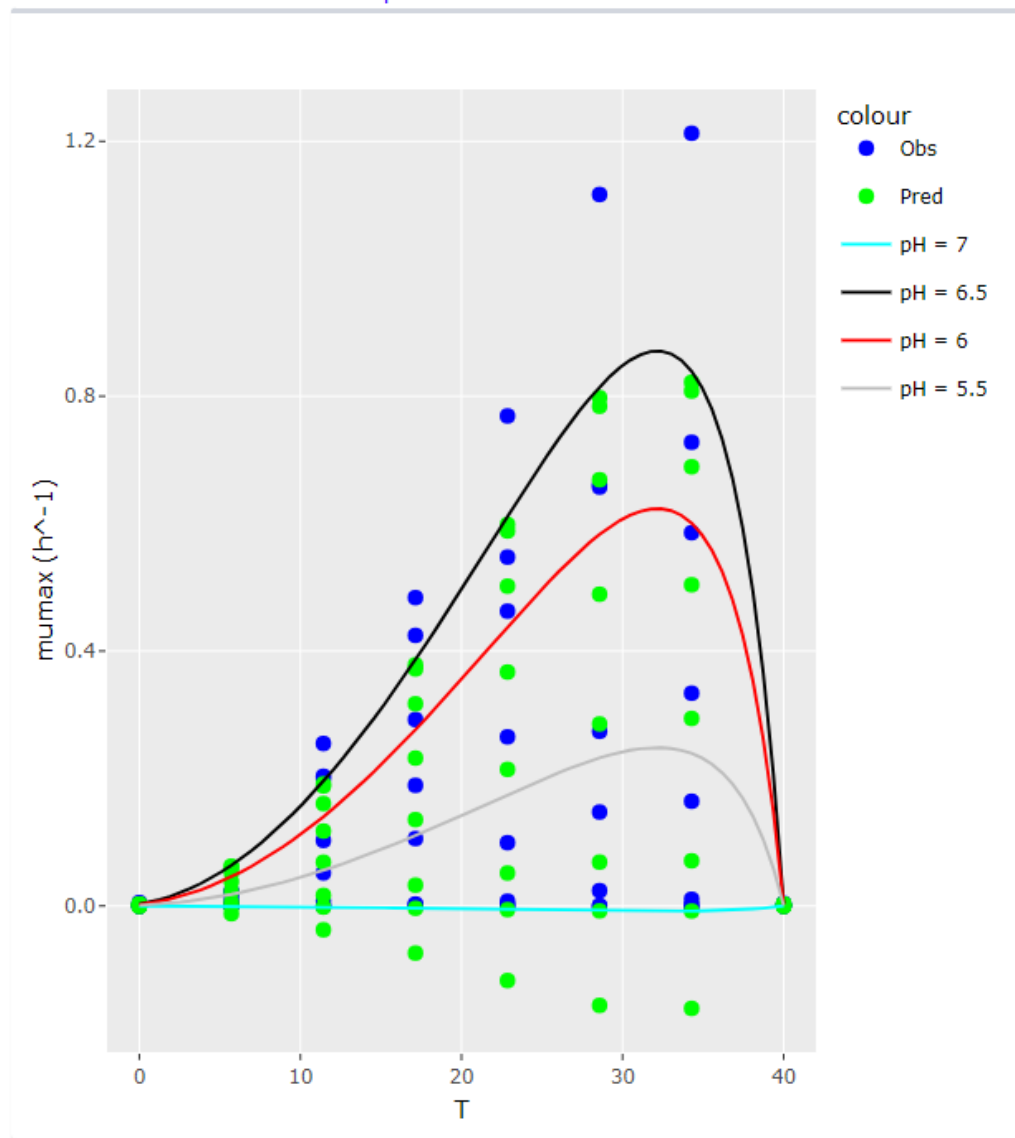


Figure 28. Updated plot of μ_{max} vs a single pre-selected variable for 4 fixed values of pH as concomitant variable.

6. Module “Primary model fitting”

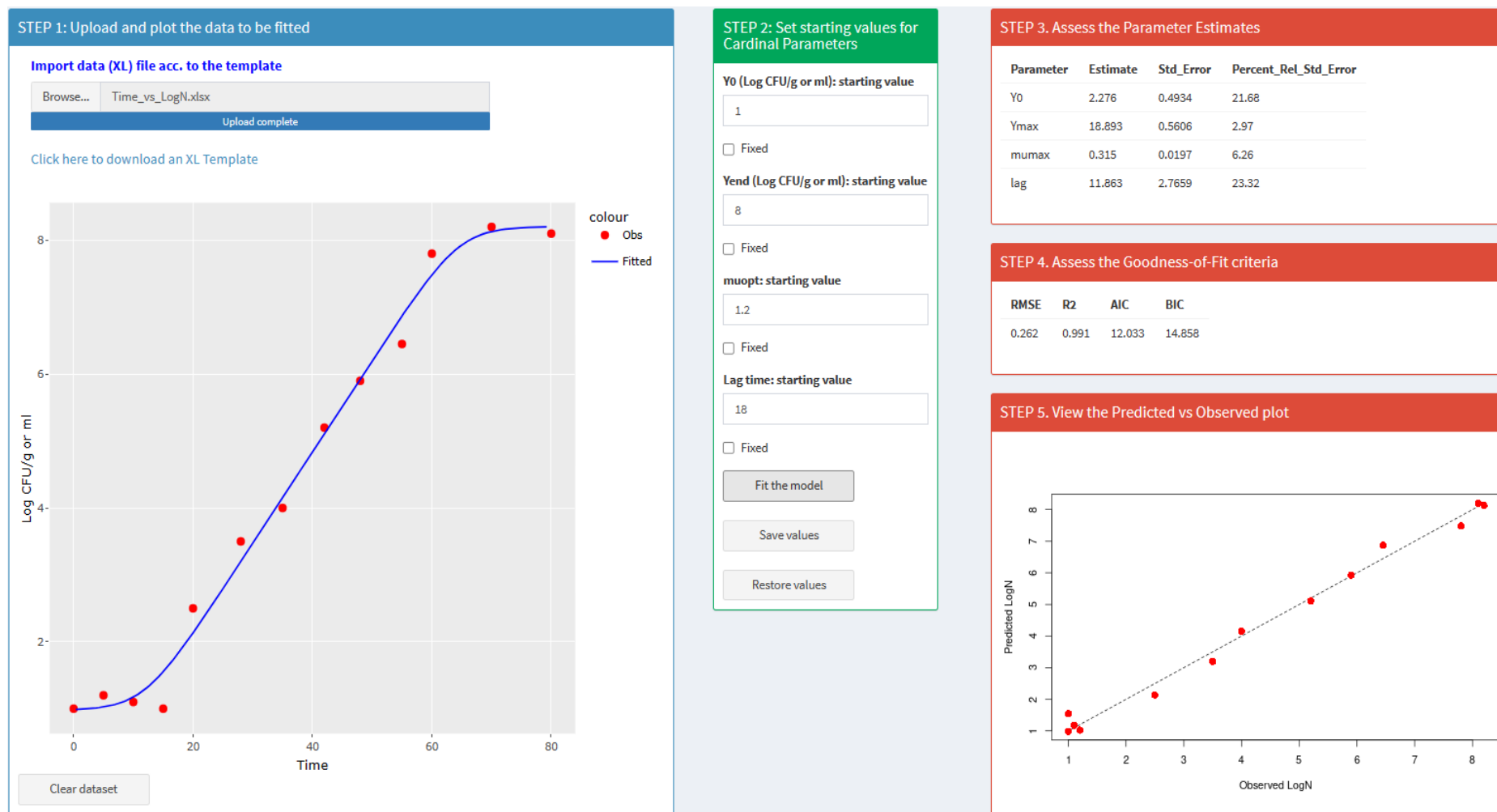
This module is used to fit the analytical (explicit) version of the Baranyi primary model (equation 15 re-typed here as *equation 21*) to user-uploaded datasets describing the changes in Log CFU/g or ml (dependent variable) of a microorganism as a function of time. The dataset to be fitted are uploaded in the form of an **XL file** or **manually typed/pasted in a table** provided by the tool.

It operates the same way as the previous fitting module for the secondary gamma model fitting, with only 4 parameters and time as the single independent variable. The following Figure provides a typical example of the output configuration of this module, which is very similar to module 5 (Figure 29).

$$\ln(N_t) = \ln(N_0) + \mu_{\max} A(t) - \frac{1}{m} \ln \left(1 + \frac{\exp[m\mu_{\max} A(t)] - 1}{\exp\{m[\ln(N_{\max}) - \ln(N_0)]\}} \right) \quad \text{equation 21}$$

$$A(t) = t + \frac{1}{v} \ln \left(\frac{\exp(-vt) + q_0}{1 + q_0} \right), \quad \text{where } v = \mu_{\max} \quad \& \quad q_0 = \frac{P_0}{K_p} = \frac{1}{\exp(h_0) - 1}$$

$$\text{lag} = \lambda = \frac{h_0}{\mu_{\max}}, \quad \text{where } h_0 = \ln \left(1 + \frac{1}{q_0} \right)$$



7. Troubleshooting

An error message as “*Error: An error has occurred. Check your logs or contact the app author for clarification.*”, may appear either in the graphical output boxes or in the Table outputs, under any of the following circumstances:

1. Under dynamic time-T or time-T/pH/aw conditions, when introducing wrong values for time interpolation intervals, as described in **page 16** of the present guide.
2. In the module “*Imported e-Platon file*”, when the maximum number of products loaded in the XL is exceeded during selecting a specific product to plot, i.e., 1, 2, 3, ..., etc. Please refer to **page 27** of the present guide for more details.
3. Whenever an input box is left blank (even temporarily, while deleting and re-typing a value), or improper punctuation is used. To avoid this, please check your keyboard language.
4. When a non-valid, i.e., non-compliant, value is used as input to a variable, in relation to the expected range of values for this variable. The eligible range of values is specific to each variable and depends on biological constraints and the relative magnitude of cardinal values (when referring to the gamma secondary models) used for the simulations. For example, h_0 cannot be negative (as lag time cannot be negative), total prevalence is not allowed to be 0, or the minimum temperature for growth (T_{min}) cannot be higher than the maximum (T_{max}) or the optimum (T_{opt}) temperature for growth, etc. It is recommended that the user remains alerted for mistyping or misuse of values for the input variables, i.e., use of values with no biological significance.

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