Microbial lag calculator

The manual for the Shiny-based application to calculate microbial lag phase duration.

The application allows to estimate microbial lag phase duration out of the user-provided growth curve dataset.

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1. UPLOADING THE DATA

The accepted file formats are csv and txt. The dataset must contain two columns (Fig. 1):

The first column: time (preferably in hours)

The second column: population size (preferably in CFU/mL).

The population size is recommend to be measured by biomass or CFU values instead of raw absorbance, This is because the correlation between CFU value and absorbance is rarely linear. However, if you're unable to provide CFU/biomass values, the calculator will also work for raw absorbance data.

The data also needs to be blank corrected and represent the alive microbial population. If a substantial part of the population is dead or senescent, the lag calculation will be affected.

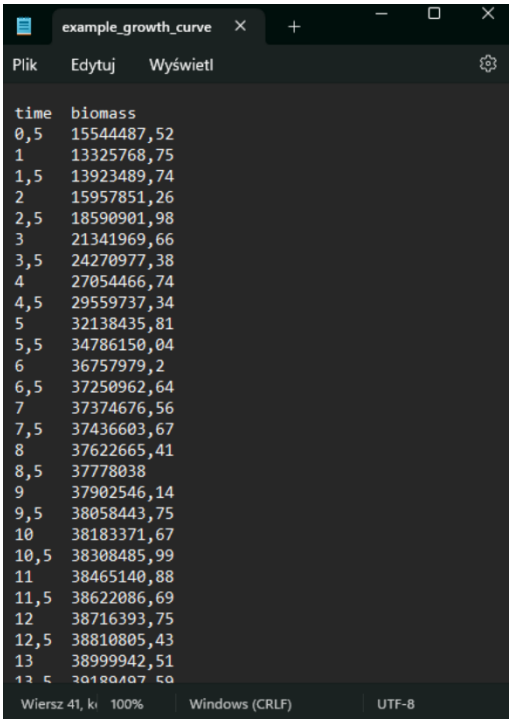

Fig. 1. Example dataset in txt format, consisting of two columns: time and biomass.

Example data can be found here:

https://github.com/bognabognabogna/microbial_lag_calculator/tree/main/shiny_app/lag_calulator/R

After uploading your dataset, specify the column and decimal separators (Fig.2). If the data is uploaded correctly, you will see the first 10 rows of your dataset displayed below.

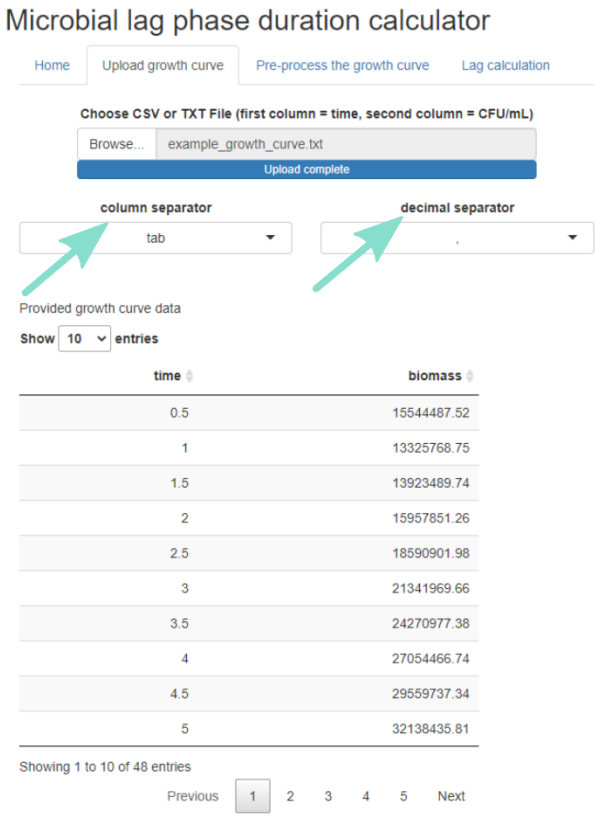

Fig. 2. Uploading the dataset. Arrows point to column and decimal separators that need to be specified.

2. PRE-PROCESSING

Your dataset will be plotted automatically with the log10 transformation applied to the biomass values (y-axis). The example data have a biomass drop at the beginning of the curve, which should be smoothen. The population reaches stationary phase after ~7 hours, and the curve is flat afterwards. Therefore, the pre-processing applied may include cutting the data after 12h and smoothening the curve (Fig. 3). Exact values can be also adjusted to improve fit of given lag calculation method.

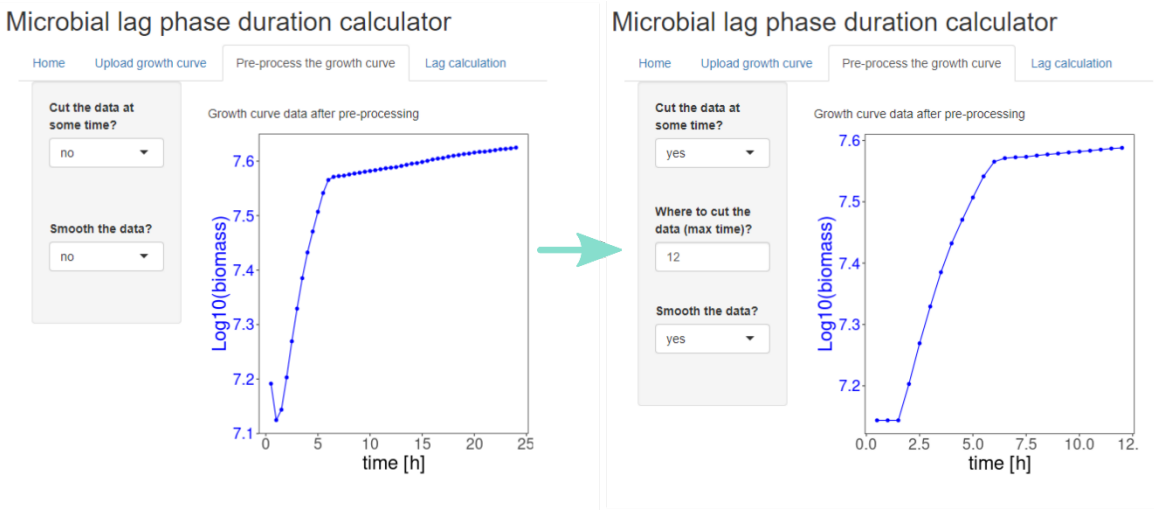

Fig. 3. Pre-processing applied to the example growth curve.

3. CHOOSE LAG CALCULATION METHOD AND ADJUST PARAMETERS

There is one Global parameter that can be changes, namely whether the initial inoculum size (biomass) is assumed to be equal to the first measurement or to the minimal value. In the example data, after the smoothening pre-processing both first and minimal values are equal. Fig. 4 shows how first and minimal values differ on not-pre-processed example data.

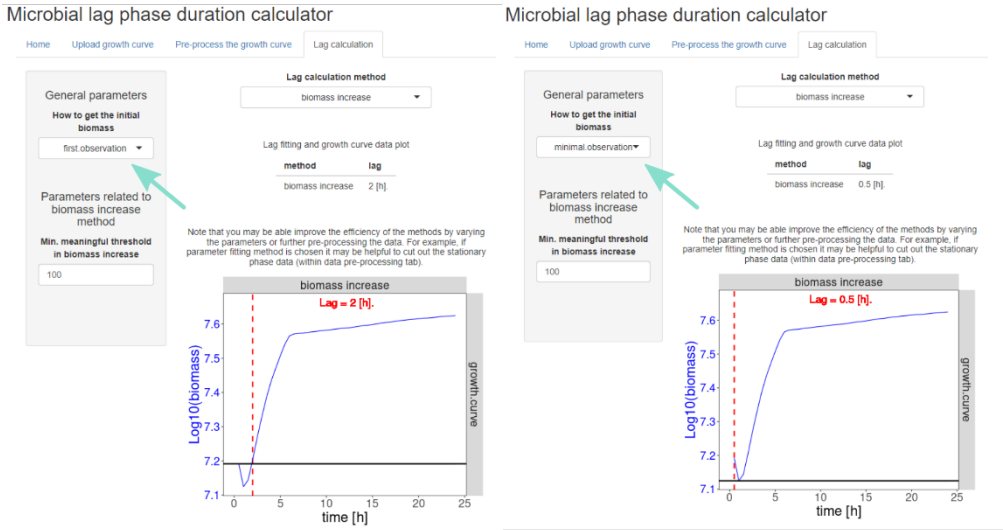

Fig. 4. The difference between first and minimal value taken as the initial inoculum size on the not pre-processed example data

There are four lag calculation method available within LAG-NIFICENT application:

BIOMASS INCREASE - the lag phase end is a point when the biomass (or absorbance) increased from the initial value by some predefined threshold (usually set as minimal detectable increase). You can specify the threshold that suits your dataset (Fig. 4). Note that if you use raw absorbance data instead of biomass values, this threshold value must be changed, we suggest to try 0.01 value.

MAX GROWTH ACCELERATION - the end of lag as the point of the growth curve where the second derivative of the population size in time is maximal (Fig. 5). The value of the second derivative of the population size is plotted as the green line.

TANGENT METHOD - the intersection of the initial density (inoculum size) line and the line tangent to the part of the curve where the growth rate is maximal (Fig. 6). The tangent line is plotted as the green line with marked point or points that correspond to the maximal growth rate.

PARAMETER FITTING TO A MODEL - uses fitting procedures to simultaneously fit all growth curve parameters (e.g. lag phase length, maximal growth rate, and maximal population size) of experimental data to a mathematical model (Fig. 7). There are two models: logistic and Baranyi.

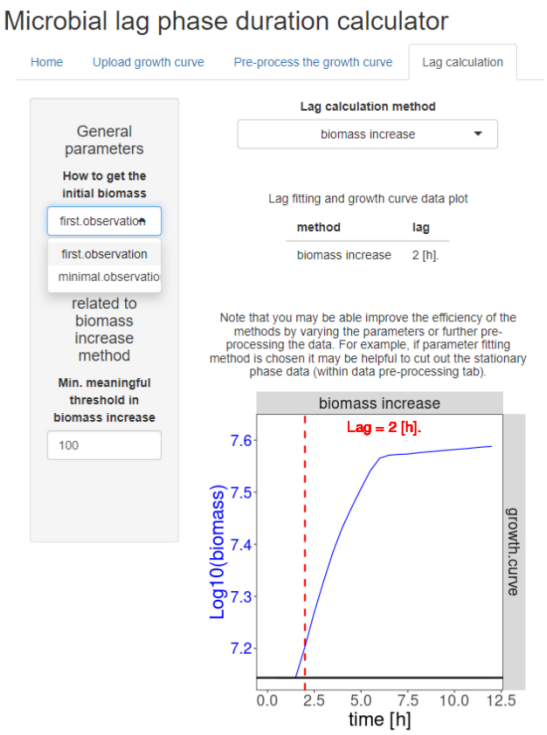

Fig. 4. The lag phase length estimated by biomass increase method

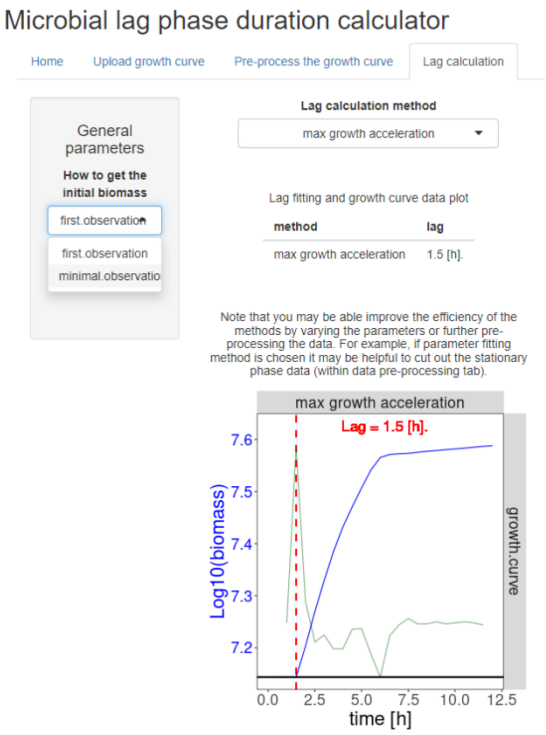

Fig. 5. The lag phase length estimated by max growth acceleration method

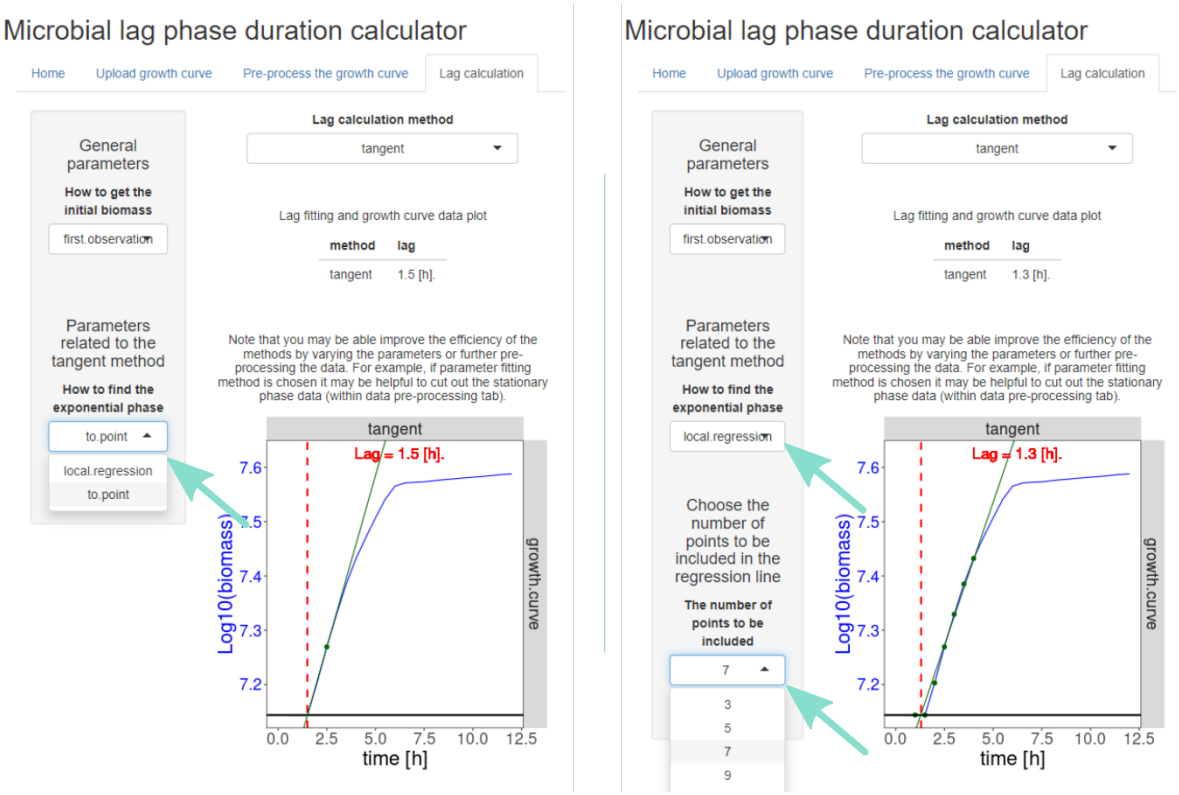

Fig. 6. The lag phase lengths estimated by tangent method, using a point where growth rate is maximal to draw a tangent line (left panel), or 7 points around the point where growth rate is maximal (right panel)

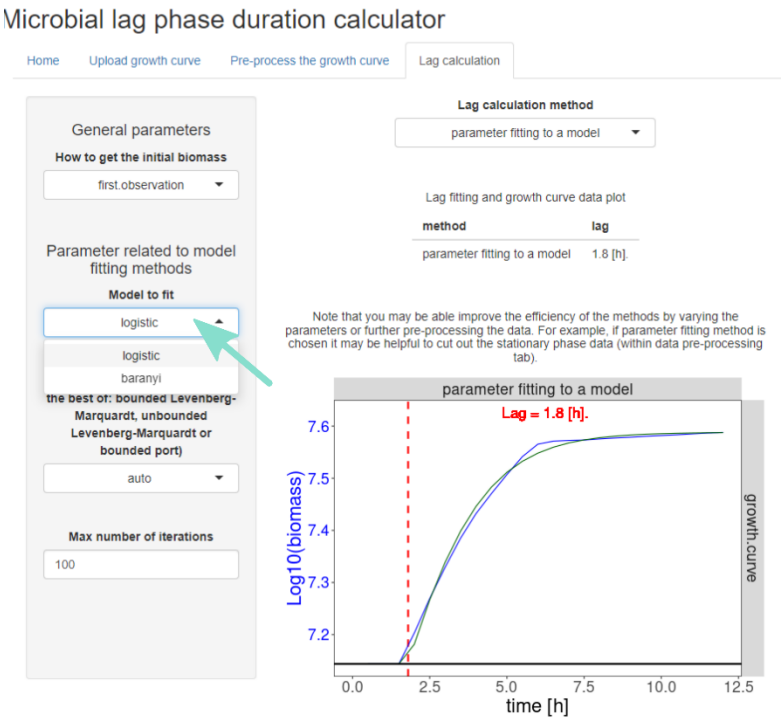

Fig. 7. The lag phase duration estimated by parameter fitting to the logistic model.