SHORT GUIDANCE FOR A TRAINING ON THE FERMENTER TOOLSOFTWARE AND SOME EXPLANATIONS TO INCREASE ECONOMIC EFFICIENCY IN A FERMENTATION PROCESSES.

1. INTRODUCTION.

Our Fermenter Tool software will help you solve the following tasks:
a. modeling of your fermentation process data;
b. predicting of the optimal processes;
c. detecting of the limiting substrates according to 'a' and then to do all the calculations to achieve forecasts 'b'.

The program for the training is designed for engineers and scientists in the microbiological industry and R&D areas for planning, analysis and control of the fermentations, as well as for undergraduate and graduate students who are trained in the microbiological field.

Two positions for the software www.fermentertool.com/tool are given to consider:

A. Regularities of a biomass growth as S-shaped curve for a Growth Inhibition Phase, GIP, Slow phase;
B. Regularities of a synthesis of products and consumption of substrates in the GIP phase of the biomass growth curve.

An Integrated Mathematical Model of Development (IMMD) is the basis of these calculations. The IMMD presents paragraphs A and B as 2 basic paradigms, which are expressed in the form of differential equations:

1. For biomass:

$$d^n X^{div}/d((X^{st}))^n = \{(K/A^2) * [(-1)^{(n-1)}] * n!\} / [(X^{st})^{(n+1)}] - C, (1);$$

2. For products (substrates):

$$dP(or - S)/d\tau = k^{div}(\text{for P or -S})X^{div} + k^{st}(\text{for P or -S})X^{st}, (2).$$

The software also provides some parameters for the Logarithmic Growth Phase, LGP. But, to get acquainted with them it is better in our article: http://iopscience.iop.org/1758-5090/3/4/045006/

In this article the positions A and B are represented by the corresponding integral equations, respectively for A this is an integral equation (10) (and also (8) (9), (45)); and for B these are integral equations (20) and (21), respectively, for products, P, and substrates, S. It is deliberately not presented these integral equations in this manual, because everyone can look at them in the article, but it will be given below pictures, that you can see in a microscope or a schematic for understanding of all these equations. Also in the section **Notation** you could find the physical meaning of all parameters.

2. MORE DETAIL CLARIFICATIONS ABOUT PARADIGMS 1 AND 2.

Concerning paragraph 'A'-paradigm #1:

It was proposed to consider a population of cells in two major age states, dividing cells and nondividing cells. For eukaryotes non-dividing stage will be stages G0 and G1 and for prokaryotes it will be stage B. Cells found in all the other stages of the cell cycle we will be considered as the stage of dividing cells. Cell population distribution thus reflects the real situation at any limitation during cultivation.

In such conditions, the cells in dormant phase, or stable cells, use a substrate (use the energy) just to maintain their lives, while the cells found in all the other cell cycle phases and called by us as dividing cells, use a substrate and energy to continue the development or the formation of new cells.

The amount of dividing cells and non-dividing cells is the total biomass.

Such a division of cell population into dividing and stable cells and the corresponding separate calculation of their energy requirements leads to the creation of a new mathematical model describing the dynamics of the behavior of cell population when grown in a bioreactor, and explains a lot.

Fig.1.An example for the simplest case as Prokaryotes:

$$X^{st} - \text{position 1, 5, 6}; \; X^{div} - \text{position 2, 3, 4}; \; X = X^{st} + X^{div}$$
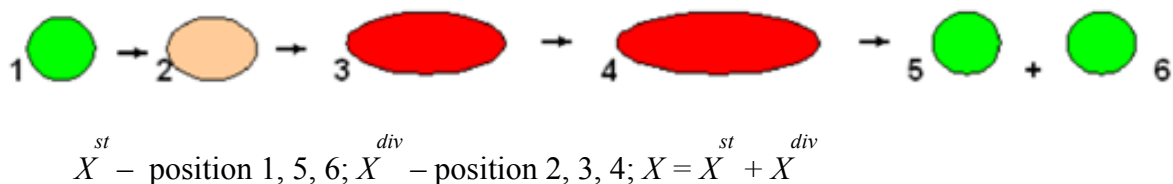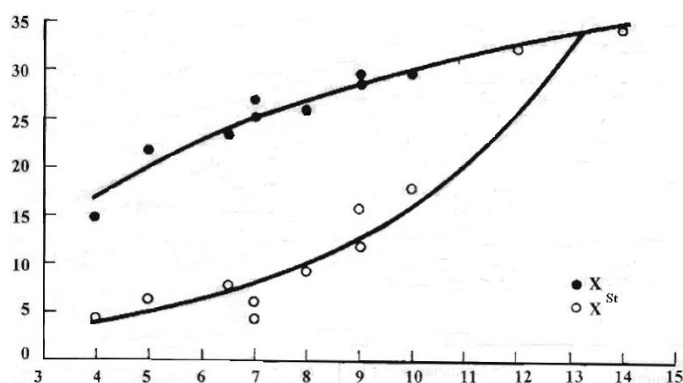
In reality we will observe in a microscope a mixture of all states from 1 to 5 and 6:

Fig.2. *Y. pestis* EV cells images were increased by 15,000 times.
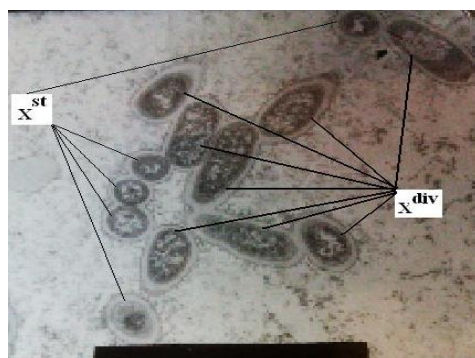
Moreover, the composition of this mixture will change with time t in the direction of monotonic increase of states 5 and 6:

Fig.3. Growth time, hours, is the abscissa axis. X, 10^9 cells/ml, is the ordinate axis.

Here the black points are the total biomass, X, and the non-colored points are the concentration of stable non-dividing cells, X^{st}. As we shall see later in the GIP phase the number of non-dividing cells always increases exponentially for all microorganisms and cells.

The same can be seen in the works of other authors on examples of cultured cells of higher animals:

Fig. 4. Data is provided with permission of Dr. J. Tyson and co-authors, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA:
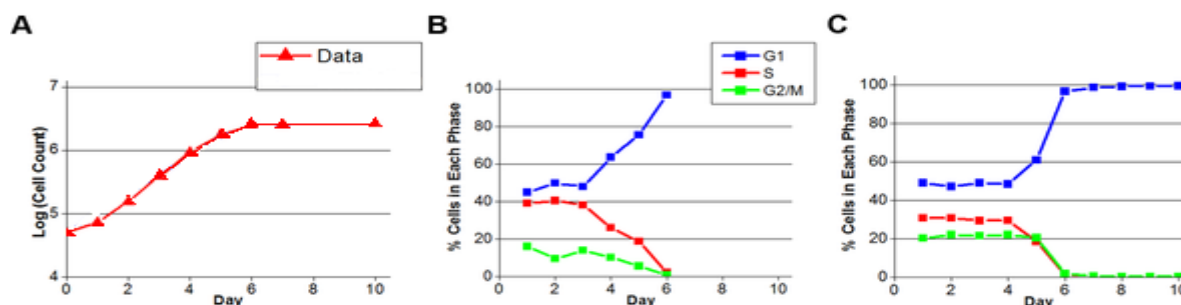

Concerning paragraph 'B'-paradigm #2:

  One of the main provisions in our experience could be formulated as: any biosynthesis involves in fact two processes - "positive" and " negative ". It is assumed, that metabolites are synthesized only by proliferating (dividing) cells. Non-proliferating (non-dividing, 'stable' or zero-age cells) cells, as a rule, destroy these products. Therefore, the signs of the constants for metabolite synthesis and degradation are opposite. The same should be stated for any substrates utilized for cell construction. All these can give a "separation" of these two processes and make recommendations to increase economic efficiency.
  Our mathematical model gives to physiological similarity of cultures in different volumes, as a criterion for different scale up procedures. This similarity is equal or close to ratio between of the dividing cells and non-dividing cells numbers for two (three, etc.) various volumes, which will be compared between each other: 10 liters, 1 cubic meter, etc. This indicator is marked with "R" in our articles.

An important feature of the IMMD theory and Fermenter Tool software is the fact that they do not make math separations to describe products and substrates but consider these categories simply as substances.

  When computing products and substrates the first step in any case is to calculate the biomass. Below in Figure 5 the biomass growth curve of plant cells in the bioreactor is shown. Further even lower in Figures 6 and 7 are the curves of product accumulation and substrate utilization. (Data was borrowed from http://barc.ernet.in/publications/nl/2000/200010-04.pdf). The yellow region is the exponential part of the biomass growth, LGP; The green area is the phase of slow growth, GIP.
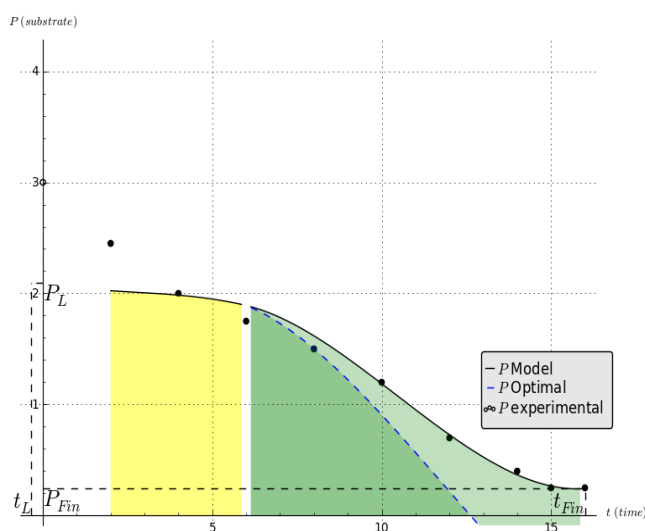
Fig.5. Growth of plant cells in bioreactor.
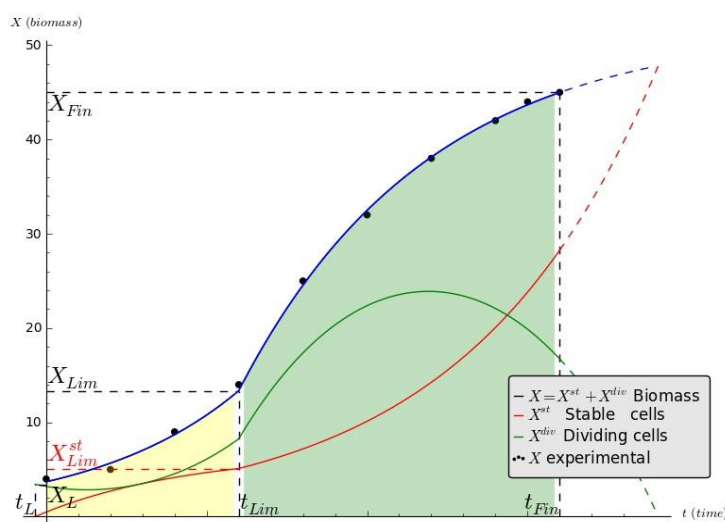
Fig. 6. Product accumulation for plant cells at their growth in bioreactor.
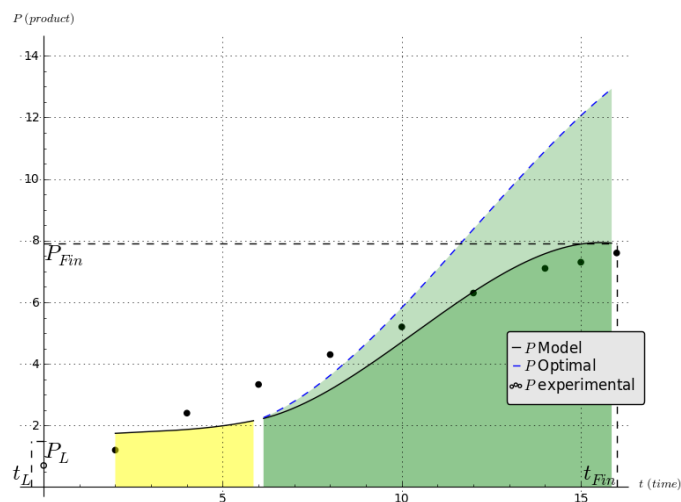
Fig. 7. Substrate utilization for plant cells at their growth in bioreactor.

In Figure 6 we see that the dashed line shows, that the product can accumulate at significantly higher concentrations, if the culture medium is balanced and if the negative contribution of non-dividing cells is reduced to zero. (The rate of utilization of the substrate, Figure 7, in this case does not interest us from this point of view and therefore we will not dwell here on this fact.)

However, the results obtained in Figure 6 this is a moment of reflection and our theory will make the calculation for the amount of make-up to achieve maximum results on the dotted line. This part of the software is not ready yet but we are ready to do this manually when carrying out the corresponding R&D works.

3. POSSIBILITIES OF THE FERMENTERTOOL TO INCREASE ECONOMIC EFFICIENCY IN A FERMENTATION PROCESSES.

Features for a biomass as a target product in the form of living cells.

The input data are the biomass concentration; it can be expressed in any units, as well as the concentration of products and substrates. If the substrates are added during the process, you should summarize their original values and to make the entering into the software with the amounts received by this way in the product field (not in the "Substrate" field in the software), and bearing in mind not the concentration, but the numbers of the substrates that was already consumed up to this process time.

A typical S-shaped growth curve of biomass is shown in Figure 1 below:

Figure 8. A typical S-shaped growth curve of biomass. The units are arbitrary.
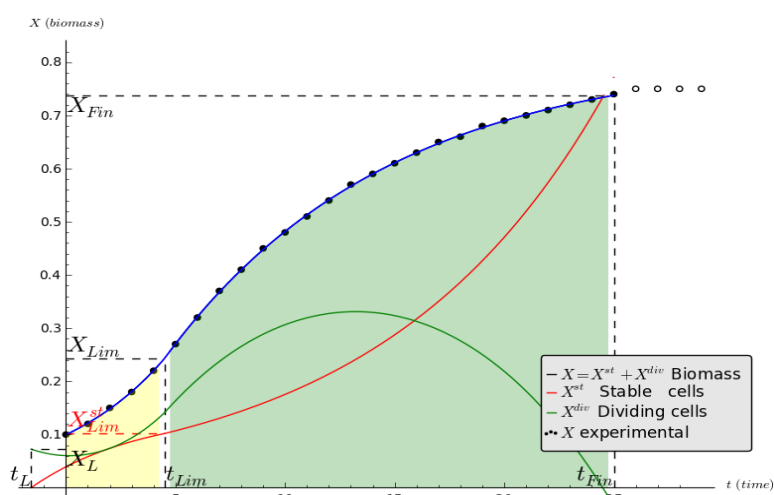
The blue dots is an experimental values of biomass, Xexp. The blue curve is a model for biomass, X. Unpainted dots denote points that were not taken for computation. The red line is a model calculation for non-dividing cells concentration, cells of zero age state, X^{st}. The green line is a model calculation for concentration of dividing cells, X^{div}.

To optimize the release of living cells at the obtaining of vaccines, probiotics, yeast $R = X^{st}/X$ was used as the most important parameter. It is the percentage of non-dividing cells. For example, if we do not take into account this indicator, workers are often finishing the processes before that time, when all cells was divided and $R_{optimal} = 1$. In this case, we are often obtaining the picture as we can see on Figure 9.

Figure 9. On an unbalanced medium the cells could not complete the proliferating process to the end.
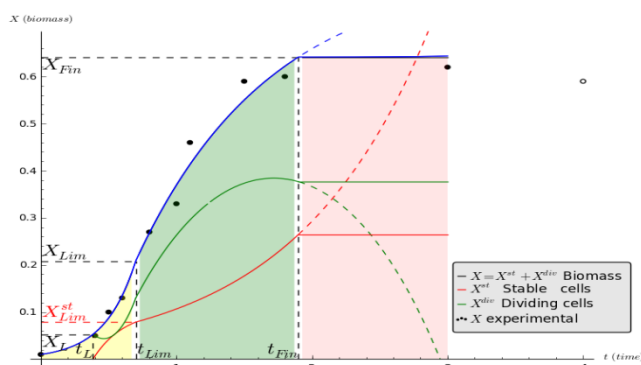
The pink region of the growth curve is the region of the stationary phase when cell growth is impossible and a mixture of dividing and non-dividing cells is present in the nutrient medium.

Balancing of the nutrient medium will allow to do further accumulation of the cells, which are resistant to external influences to continue –it is the pink dotted line on the Figure 9.

Features for a biomass as a target product in the form of living cells And as an active "metabolic agent".

Manufacturers of starter cultures can control two qualities of their final product. This is the rate of fermentation and ripening time, for example, in the production of lactic acid products.

Significant economic benefits reaching 20 percent or more can be, if you use this software in this field. You can get starter cultures with a very high metabolic activity but with a low R value (this is the fraction of non-dividing cells that survive with lyophilic drying). Or vice versa, with lower values of metabolic activity,but with a higher R.

This is shown in Figures 10-13 below.

**Figure 10. Forecast # 1. Ripening time , t process,   % from maximal value,from cultures, that were taken at different times of fermentation, % from maximal value.**
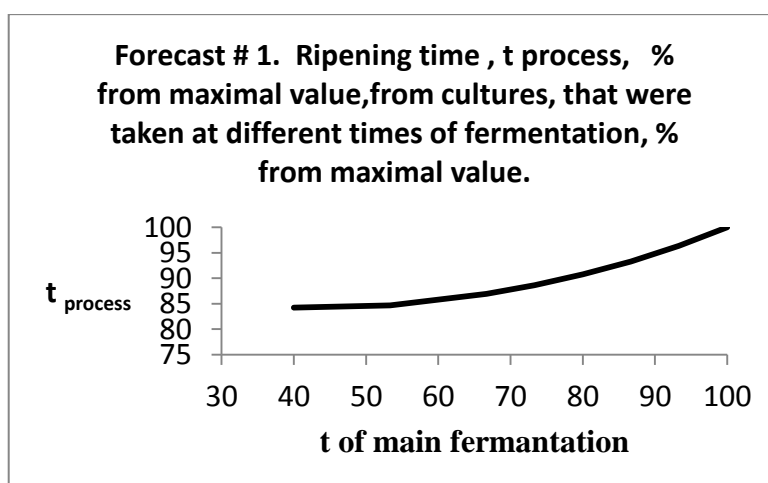

Figure 11. Forecast # 2. The rate o pH changes for each 0.5 hours, % from maximal value, from cultures, that were taken at different times of cultivation in fermenter, % from maximal value.
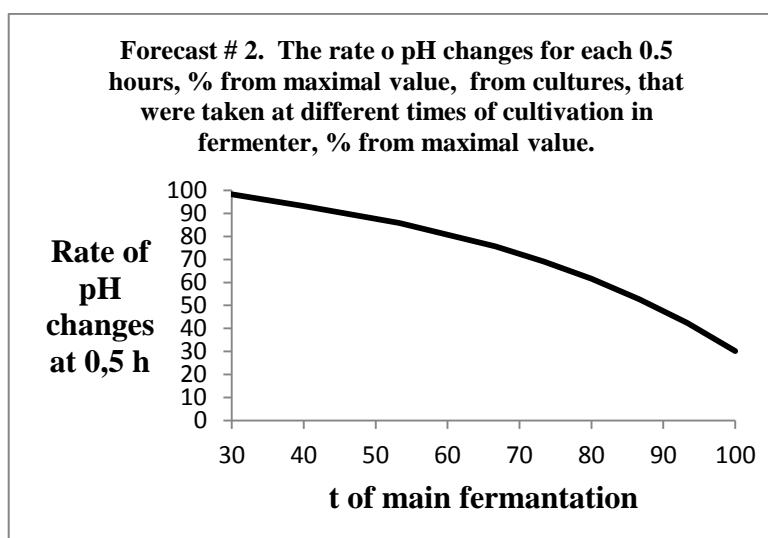
Figure 12. Forecast #3. Ripening time from cultures, t $_{process}$, % from maximal value, taken at different R values during the growth curve.

Forecast #3. Ripening time from cultures, t $_{process}$, % from maximal value, taken at different R values during the growth curve.
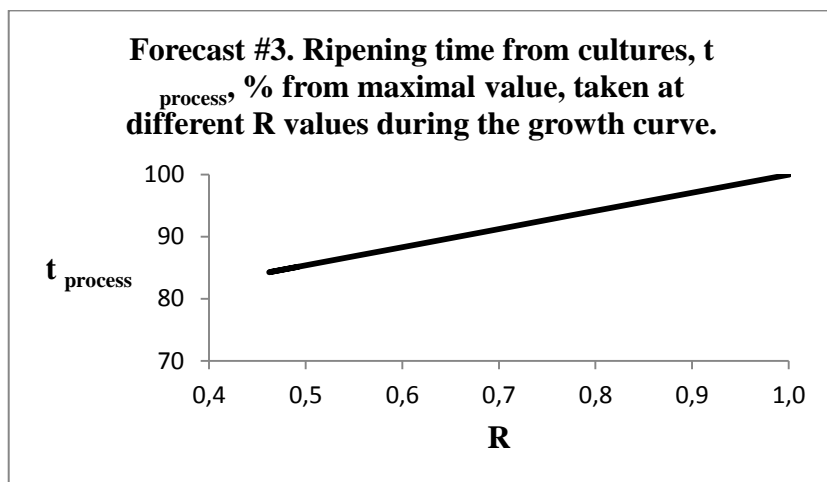
Figure 13. Forecast #4. The rate o pH changes for each 0.5 hours, % from maximal value, from cultures, that were taken at different non-dividing cells concentrations in a fermenter, X^{st}, % from maximal value.

Forecast #4. The rate o pH changes for each 0.5 hours, % from maximal value, from cultures, that were taken at different non-dividing cells concentrations in a fermenter, X^{st}, % from maximal value.
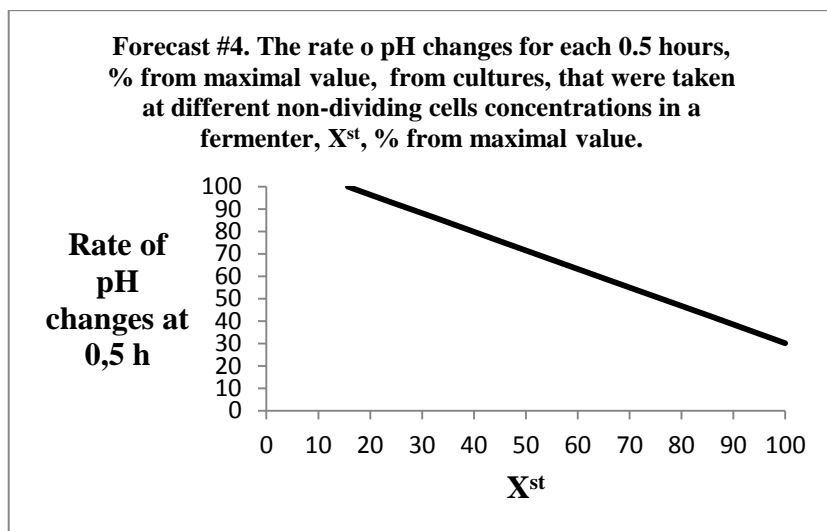
Use of the software to prevent uncontrolled destruction of the target product on poorly balanced nutrient media.

We carried out similar work on a very wide range of microorganisms and products. The following is an example of the antibiotic gramicidin S. This work was carried out before the creation of this software but using the IMMD theory.

Figure 5. The calculated and experimental data on the cultivation of Bacillus brevis for gramicidin S shows. The productivity of 1.5 times by optimization using the IMMD theory has increased.
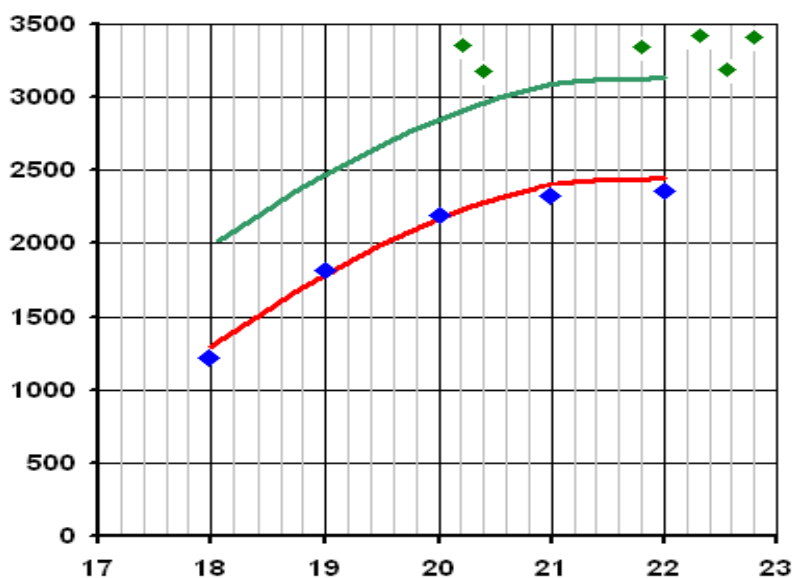
The ordinate shows the concentration of antibiotic, mg/l, and the abscissa is a cultivation time in hours. Blue points are experiments on an unoptimized nutrient medium. The red curve is a model for these experiments.

The green curve is a forecast for unoptimized nutrient media, if we exclude the destruction of the product by non-dividing cells. Green points are the realization of a favorable forecast.

This software as mentioned above also can give parameters for the calculation of nutrient feeds for the realization of a favorable forecast.

4.PROCEDURE FOR THE USE OF THE SOFTWARE AND REQUIREMENTS FOR THE GUARANTEED OBTAINING OF GOOD RESULTS OF THE ANALYSIS OF YOUR DATA.

1) Enter your login and password.
2) Select the button, which is necessary for calculations. This can be "Calculations for biomass" or "Calculations of biomass and product"; the last button will do the calculations also for substrates. You should remember that our software can work only with concentrations data. For biomass: g/l, billions of cells/ml, a.u./ml.
3) Enter into the left column the values of the time of cultivation, into the right one the values of biomass.
If you have done the selection buttons Calculations of biomass and product", you can enter the values of products and substrates after biomass in the field, which lies on right from the biomass column.
For one of biomass you can make putting up to 10 values of different substrates (e.g., sources of carbon, nitrogen, phosphorus, magnesium, etc.) and up to 10 different values of products (e.g., penicillin and/or penicillin biosynthesis related products).
4) Push "Send" button, when the procedure of entering of your data was completed; and you will be waiting for the Report in your Inbox for your emails during from 1-2 hours to 1-2 days, because the software can not be included at any time of the day.

Please note:

1) The data for the biomass should be as S- shaped curves (!)

2) The software minimal quantity of dots for the Biomass,X=f(t), should be equal no less than 8 , (~4 dots for Exponential Phase of the growth curve and ~4 dots for Slow Phase.)

3) The stationary growth phase (also lag- and death phase) has a little significance for our software. If you have doubts about your data, please, check them through charting in the Excel program. Use a stationary phase, if the biosynthesis of your target product occurs in this phase of growth.

4) If you use large numbers (millions or billions) it is better to keep millions and billions in your mind and use simple quantities. For example, 400 000 000 is better to place into the software as simple number 400 ... but, we are keeping 'millions' in our mind.

5.NOTATION

LGP logarithmic growth phase; **GIP** growth inhibition phase;

S substrate concentration, g l^{-1};
X biomass concentration, OD units or g l^{-1} or other;
OD unit of an optical density;

τ time, hour, or other;

P products , units P ml^{-1};
dP/dτ absolute rate of product synthesis, units

of P(ml h)$^{-1}$;
Q=−dS/dτ absolute rate of substrate consumption;

QO$_2$ oxygen mass exchange rate, mmol
O$_2$/(volume units per time units);
J stochiometric factor of energy substrate

(**S**) oxidation, Joule of S/mmoleO$_2$;
μ specific growth rate of biomass X,

(hour)$^{-1}$;
q = Q/X or

q = (1/X) ∗dP/dτ specific rate of substrate utilization

or product synthesis, units of **S**(units

of **X** h)$^{-1}$ or units of P(ml units of
X h)$^{-1}$;
a trophic coefficient, amount of energy

substrate consumed for the synthesis of

a biomass unit, Joule of **S**/Joule of **X** or

units of **S**/units of **X**;

f amount of energy substrate accumulated

in biomass **X** during cultivation on a

synthetic medium, Joule of **S**/Joule of

X or units of **S**/units of **X**;

m energy maintenance coefficient, the

rate of substrate consumption for

maintaining viability of one biomass

unit per a unit of time, Joule of **S**(Joule

of **X** h)−1 or units of **S**(units of **X** h)$^{-1}$;

A = m/a (1) Parameter describes a delay of the

biomass growth rate; (2) specific rate of

accumulation of stable cells, h^{-1};

X$_p$ maximum biomass concentration, when
all the energy generated during

cultivation is consumed for cell viability

maintenance;

X$_{Lim}$ biomass concentration in the end of
exponential growth phase and beginning

of growth inhibition phase;

Xst concentration of the biomass of zero age
cells (stable), the content of resting cells,

OD units or g l^{-1};

X^{div} = X − Xst concentration of proliferation biomass,
OD units or g l^{-1};

τ$_{Lim}$ time of exponential growth phase
termination, hour;

X$_{Lim}$st concentration of stable cells at the end
of exponential growth phase, **OD** units

or g l^{-1};

R ratio of Xst to biomass X, relative

content of stable cells in the biomass,

synchronization degree, part of 1;

X$_l$ initial biomass concentration in LGP
corresponding the beginning of population

structuring, OD units or g l^{-1};

X$_{final}$ final biomass concentration, at which
R = 1 (when energy consumption is

limited);

k$_p$^{div}, k$_p$st, k$_s$^{div}, k$_s$st constants of metabolite
and substratebiochemical reaction rates, [units of **P**

(or **S**)/(ml∗ units of biomass **X**} per one

hour)];

P$_{Lim}$ metabolite concentration or activity
at the end of **LGP** and

beginning of **GIP**, units of **P** ml^{-1};

P*l* metabolite concentration or activity in **LGP**, when biomass structuring occurs

at $\mathbf{X} = \mathbf{X}_l$, units of **P** ml^{-1}.

Best regards,

Sergey Klykov,

August, 8, 2017.