Numerical Analysis of Factors which Influent the Biotic Systems Using the Ferment Activity of Beer Yeast

Mariana R. Milici, Rodica Rotar, L. Dan Milici

***Abstract*—**The beer producing is one of the eldest technological process which uses the beer yeasts to transform the fermentable glucides into ethylic alchool, carbon dioxide and aroma compound. The alive cells are open sistems, separated by environment through the cytoplasmatic membrane, and them physiological state is determined by controled transport of nutritives to the inside of cell or of the metabolism products to the outside of cell. The study proposes to find the most efficient way to grow the intracellular trehaloze content through beer yeast suspending into trehaloze solutions by different concentrations, at different thermo-stating temperatures and in different contact times, taking into account that this technique allows the passive transfer of exogenetic trehaloze inside the cells both at a new propagated cell population, and at cells resulted from an industrial inoculums.

***Keywords*—**approximation methods, factors of influent, ferment activity, numerical analysis.

I. Introduction

Qualitative and quantitative studying the passive and active electrical properties of biological systems, the phenomenological theory of electromagnetism is used, into a general wording, concerning the electrical field inside a conductive volume, generated by a certain applied current density and conventionally admitting the existence of a linear, infinite, homogenous and isotropic medium, characterized by the electro conductibility, permeability and permittivity.

The biological potential sources are situated in electrolytic medium, with complex character, whose time nonlinearity and variableness are generated by functional characteristic features of biological structures. Concurrently, the interpretation of potential difference existent between two points of the biological conductive medium requires studying the properties of whole system, in which the biological sources are in interdependence. Given the ideal formulation of electromagnetism laws, which are applied however in biological phenomena, are evident difficulties in mathematical simulation of real electrical features of the living body, which is a no homogeneous conductive volume, delimited in no uniform space, with electrical anisotropy, composed of a multitude of independent sources, which interfere spatial and temporal in variable way.

To determine the spatial distribution of electrical and magnetic fields in biological medium, it appeals to Helmholtz homogeneous equations, in which intervene complex sizes dependent on spatial and pulsation coordinates. For determination of electrical biological field intensity are taken into consideration a series of properties of conductors as: capacity, propagation, inductive, surface and "frontier" effects, as well as biophysical and biochemical effects specific to living body. The obtained values for different electrical parameters of living structures are averages achieving the exploration of the whole tissue from measurement circuit.

On the base of living matter, there are molecules, with biological structures and noteworthiness, submitted to some permanent changes by chemical reactions, and to some perturbations produced by electromagnetic radiations, taking place continuous reshufflings of biological structure organization. In case of the variation of the environmental conditions, inside a biological system, occur opposite processes, that diminishes and annihilates the action of the perturbation factor. In the living cellule occur numerous energetically transformations that have correspondent in electromagnetical energy.

Choosing of a certain beer yeast stem for beer obtaining, in specific conditions to lay in one's stock of raw materials, of endowment and of used technology, is achieved taking into account the main specific features of the beer yeast: final fermentation degree and the fermentation speed, capability to assimilate the substances which participate in metabolism, multiplication efficiency, sedimentation and flocculation capability, spectrum and quantity of secondary products of fermentation with implications in the beer taste and aroma, deterioration and contamination resistance.

The rejoinder life cycle, determined by number of complete divisions of each individual cell is genetic controlled, but

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influenced by medium conditions. After the cell divided by a certain number, enters into the cell senescence, loosing definitively the multiplication capability and finally dies. The chronological life cycle, defined as maximum outliving time of the cells maintained in the stationary growing stage and characterized by a temporary loosing of multiplication capability due to the nutrients absence, constitutes an important controlling instrument of degeneration at the macromolecular level and of cell death rate.

The study object is to evaluate in which measure the supplementation of yeast inoculums with linoleic acid succeed to minimize the negative effect of the thermal shock stress factor on the one side, and the subduing to repetitive running of fermentation cycles, on the other side.

The study proposes to find the most efficient way to grow the intracellular trehaloze content through beer yeast suspending into trehaloze solutions by different concentrations, at different thermo-stating temperatures and in different contact times, taking into account that this technique allows the passive transfer of exogenetic trehaloze inside the cells both at a new propagated cell population, and at cells resulted from an industrial inoculums.

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II. The Generation and Transmission of Electrical Signal Inside Living Bodies

The biological electrical medium has some special features, proper to living bodies. It is a nonuniform, nonhomogeneus and no isotropic medium, with numerous structural and functional differentiations, complex correlated.

The living body is, at the same time, a source of electrical voltages, generated through energy mechanisms that result by cellular metabolism, and an electrolytic conductor in which the electrical charges remove through ionic transfer.

The electrical features of biological mediums are being determinate by water and electrolytes repartition as against the active membranes. The membrane's aim in adjusting of the hydroelectrically distribution confirms the idea that the biological activity takes place at separation limit between two distinct mediums, through an active separation membrane.

The cellular membrane has a capability generated by the existence of some dielectric substances contained into the membrane structure and by the electrical charges' polarization aside and another side of its. The limited membrane's permeability in comparison with certain ions in rest state and the increasing of the permeability during the depolarization make that this electrolytic capacitor being an imperfect capacitor with important electrical losses.

The time constant of membrane is defined as product between the resistance and the capacity of membrane and represent graphically by a charge and discharge curve at a ΔV voltage.

$$\tau_m = r_m \cdot C_m \tag{1}$$

The electrical capacity of cellular membrane has value as $\mu F/cm^2$ order (from 0,5 to 1,5 $\mu F/cm^2$), at muscular cellules having the highest capacities (till 5 – 8 $\mu F/cm^2$).

The probing of the impedance in alternative current led to establishing of the real values of electrical capacity of cellular membrane. The following relation defines the transmembrane impedance:

$$Z_m = \left(C_m + jB_m\right)^{-1} \tag{2}$$

where:

C_m – membrane conductance

B_m – membrane electric susceptibility $B_m = \omega \cdot C_m$, ω being the pulsation of alternative current.

The inverse value of impedance Z_m is represented by the membrane's admittance $Y_m = 1/Z_m$. The exploring on alternative current show a negative electrical reaction in cellular structures:

$$X_c = -\frac{1}{\omega \cdot C_m} \tag{3}$$

This establishment can be interpreting in the sense that the inductive reactance is not specific to living bodies, and the capacitive reactance has a dominant value.

III. The Factors Which Influent The Beer Yeast Fermentation Activity

The fermentation activity of yeast and implicit the beer quality is influenced by three great categories of factors:

- the used yeast stem;
- the chemical composition of unfermented beer;
- the fermentation conditions.

Due to the reduced volume, the yeast cell, as all microbial cells, is extremely sensible at the variation of bio-synthesis parameters, especially at the concentration variations of nutritive substratum. The nutrient concentration increasing determines, usually, a stimulation of microbial metabolism, but only till a limit value over that the concentration increasing even remain without effect, or has a negative influence on cellular physiology through substratum inhibition or through the osmotic pressure increasing.

The fermentation conditions are very important for rapid starting and fermentation in good conditions of the unfermented beer:

- chosen propagation method;
- yeast doze chosen at impregnation, correlated with the impregnation temperature and multiplication degree during the fermentation process, correlated with the impregnation doze and fermentation duration;
- aeration and agitation;
- fermentation temperature and pressure;
- chosen fermentation duration and dimension and form of fermentation recipients;
- getting in, purification and cheeping of the yeast.

Temperature is one of the most important physical parameters, deep involved, through its effects, in the increasing of fermentation capability. The temperature variations have effect over the efficiency of substratum transformation, over the nutritive requests of the yeast and over the fermentation speed.

Repetitive re-impregnations affect the fermentation capability of beer stem because occur changes in cellular wall physiology, is affected the integrity of the membrane mitochondrial functions. Those effects extending is a phenomenon that depends on species and can be strengthened in case of using of yeast at the "high-gravity" unfermented beer fermentation, more and more used technique.

The influence factors with physical-chemical character (osmotic pressure, hydrostatical pressure, hydrodynamic detrusion force during manipulation, pH, temperature, content of oxygen, ethanol, carbon dioxide), and the nutritional influences (HG unfermented beers, required sources of carbohydrates and azote, the content of inorganic zinc and magnesium ions, of vitamins and phosphor) have a outstanding influence over the cellular growing and fermentation capability of yeast.

The study was achieved on the industrial stem izolated by production culture from S.C. Bermas S.A. Suceava – *Saccharomyces carlsbergensis* – mentained by periodical replication on malt wort with aga and keeped at 4°C under paraffin oil sterilized precursory at 170°C, 2 hours.

In case of studies directed in order to evaluate the beer yeast quality and the influence of different factors on beer yeast viability and vitality during the successive fermentations, beer yeast tests were sampled from industrial inoculums collected separatelly, according to the used generation in production.

The study method evaluates the viability of beer yeast cells from the perspective that the cells are considered died when they lost non-reversible the ability to reproduce themselves. Although it is a method which needs more time and multiple manual labours, it is more accurate than the method of vital dyeing with methylene blue or violet crystal. The method consists in indirect numbering of alive beer yeast cells by growing on dense mediums and consists in inoculation of a known volume from cells suspension on YPG solidified medium, in Petri plates and in numbering of cultures formed after a growing period in optimal conditions.

IV. Mathematical Methods for Data Processing

Because in the large majority of cases the tests direct to a real function of real variable, the approximation of this characteristic, in the specified cases, consists in the approximation of a real function, approximation named interpolation too. The approximation of the certain real function is made by simple and easy utilized functions, especially through implementation of the computing of the values of this function. Because the real function set is a linear dimensional infinite space, while the function sets in which we look for the approximation are dimensional finite spaces, in actual fact, the abstract problem that stand on the base of approximation techniques consists in replacing of one element from an dimensional infinite space by representatives of one dimensional finite space. To can specify the "approximation" notion and to can appreciate the error made through the above specified replacing, the using of "norm" mathematical concept is needed.

Considering a real linear space X, we define on it the norm symbolical noted by:

$\|\ \| : X \to \Re$, having the properties (that satisfy the axioms):

$$\begin{aligned} &a)\|f\| \geq 0, \forall f \in X \\ &b)\|f\| = 0 \Rightarrow f = 0 \\ &c)\|\alpha f\| = |\alpha| \cdot \|f\|, \forall \alpha \in R, \forall f \in X \\ &d)\|f+g\| \leq \|f\| + \|g\|, \forall f, g \in X \end{aligned} \tag{4}$$

If on the linear space X it was defined the norm $\|\ \|$, X is a normalized linear space. On these terms we could specify the "approximation" of one function from normalized linear space X, defining what means a best approximation.

Considering the dimensional infinite functions space X, normalized by $\|\ \|$ and X_m a linear subspace of X, dimensional finite with $dim\ (X_m) = m$, we define the notion of approximation of one function $f \in X$ by function $g_m \in X_m$ considering $\|f\text{-}g_m\|$ as a measure of the error that is made if instead of the function f is used the function g_m. It is naturally on these terms to say that g_m is the best approximation of f if:

$$\|f - \bar{g}_m\| = \inf_{g_m \in X_m} \|f - g_m\| = \alpha_m \tag{5}$$

The main question which is mooted consists in the fact that from the given definition doesn't result that always is a best approximation, not that this, in case it exists, is unique. A much delicate problem, that requires approximations in its turn, is the effective computing of the best approximation in case it exists.

In many cases, in the function approximation theory is sufficient a hypothesis flimsier than the existence of one norm on the space X namely is sufficient the existence of one semi-

norm on X that is a function noted also $\|\ \| : X \to \Re$, but satisfying only the axioms *a*, *b* and *d* from (4). The existence and oneness of a best approximation is assured by the next theorems which will be enounced without demonstration.

Generally, when we know data on certain moments of time, we can find a continuous function that approximates the evolution of these data. Now there are known several kinds of approximation functions able to approximate data that have a certain evolution.

Approximation by Lagrange polynomial, uses the Lagrange polynomial $L_n(x)$ and is in the form:

$$L_n(x) = \sum_{i=0}^{n} f(x_i) \cdot \prod_{\substack{j=0 \\ j \neq i}}^{n} \frac{x - x_i}{x_i - x_j} \tag{6}$$

Newton approximation, eliminates the disadvantage of the Lagrange Polynomial which consists in fact that, in case of adding of one additional approximation node, using the divided differences expression, the approximation polynomial is written as:

$$L_n(x) = f(x_0) + [x_0, x_1]_f (x-x_0) + [x_0, x_1, x_2]_f (x-x_0)(x-x_1) + \ldots$$
$$\ldots + [x_0, x_1, \ldots, x_{n-1}, x_n]_f (x-x_0)(x-x_1)\ldots(x-x_{n-1}) \tag{7}$$

named also Newton interpolation formula.

Square average approximation. It is easy verified that will be a best unique approximation as polynomial form:

$$\overline{P}_n(x) = \sum_{j=0}^{n} a_j x^j \tag{8}$$

named discrete average approximation.

Approximatin by Spline functions. The obtained cubic Spline functions is named natural, or as the form:

$$S'_{3,0}(x_0) = f'(x_0); S'_{3,N-1}(x_0) = f'(x_N) \tag{9}$$

the functions resulted in these conditions being named strained cubic Spline functions.

Approximation by rational fractions (continuous fractions). According to the specialized literature, a continuous fraction is defined as an expression as the form:

$$a_0 + \cfrac{b_1}{a_1 + \cfrac{b_2}{a_2 + \cfrac{b_3}{a_3 + \ddots}}} \tag{10}$$

where $a_0, a_1, \ldots, a_n, b_1, \ldots, b_n$ are named the elements of the continuous fraction.

Approximation of the exponential curves. In case of some exponential curves the approximation by polynomial functions is made with high errors, reason for that there was tried other approximation methods satisfactory from the point of view of error, being obtained for these curves through using of one approximation function as the form:

$$y(x) = \sum_{i=1}^{m} c_i \cdot e^{-\lambda_i \cdot x} \tag{11}$$

with the condition $m \leq n$, therefore the number of sum terms smaller than the number of interpolation nodes.

For an array of *n* experimental data $x_1, x_2, \ldots, x_n$ there are defined:

- *Mean* is a value intended to resume the relevant features of one set of values. In this respect, it combines all set values as *arithmetic mean*:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i = \frac{x_1 + x_2 + \ldots + x_n}{n} \tag{12}$$

There are cases when the information results from the *geometrical mean* computing:

$$\bar{\bar{x}} = \left(\prod_{i=1}^{n} x_i\right)^{1/n} = \sqrt[n]{\prod_{i=1}^{n} x_i} \tag{13}$$

- *Median* is defined for a discontinuous set of *n* values as being the central value of the set if *n* is odd or the average of those two central values if *n* is even:

$$median = x_{n/2} \tag{14}$$

- *Variance* (*var*) is, in case of one array of values, the sum of squares of deviations from the mean divided to the number of values:

$$\sigma^2 = \frac{1}{n}\sum_{i=1}^{n} \left(x_i - \bar{x}\right)^2 \tag{15}$$

- *Standard deviation* (*stdev*) is the positive square root of variance, being a measure of variance of one distribution for one sample of values:

$$s_x = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n} \left(x_i - \bar{x}\right)^2} \tag{16}$$

- *Covariance* (*cvar*) is a measure of linked viability of two variables or sets of data $x_1, x_2, \ldots, x_n$ and $y_1, y_2, \ldots, y_n$ around of them mean. It obtained as sum of deviations of those two sets of values given those mean.

$$c\,\mathrm{var}(x, y) = \frac{1}{n}\sum_{i=1}^{n} \left[\left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)\right] \tag{17}$$

- *Corelation coeficient* (corr) is the measure of interdependance of two sets of data $x_1, x_2, ..., x_n$ and $y_1, y_2, ..., y_n$:

$$corr = \frac{\sum_{i=1}^{n}\left[\left(x_i - \bar{x}\right)\left(y_i - \bar{y}\right)\right]}{(n-1)\cdot s_x \cdot s_y} = \frac{n\sum_{i=1}^{n}(x_i \cdot y_i) - \sum_{i=1}^{n} x_i \cdot \sum_{i=1}^{n} y_i}{\sqrt{n\cdot\sum_{i=1}^{n} x_i^{\,2} - \left(\sum_{i=1}^{n} x_i\right)^2} \cdot \sqrt{n\cdot\sum_{i=1}^{n} y_i^{\,2} - \left(\sum_{i=1}^{n} y_i\right)^2}} \quad (18)$$

The multiple corelation coeficient is evaluated between the real values of the set of data and the values estimated by regression.

V. Experimental Data Processing

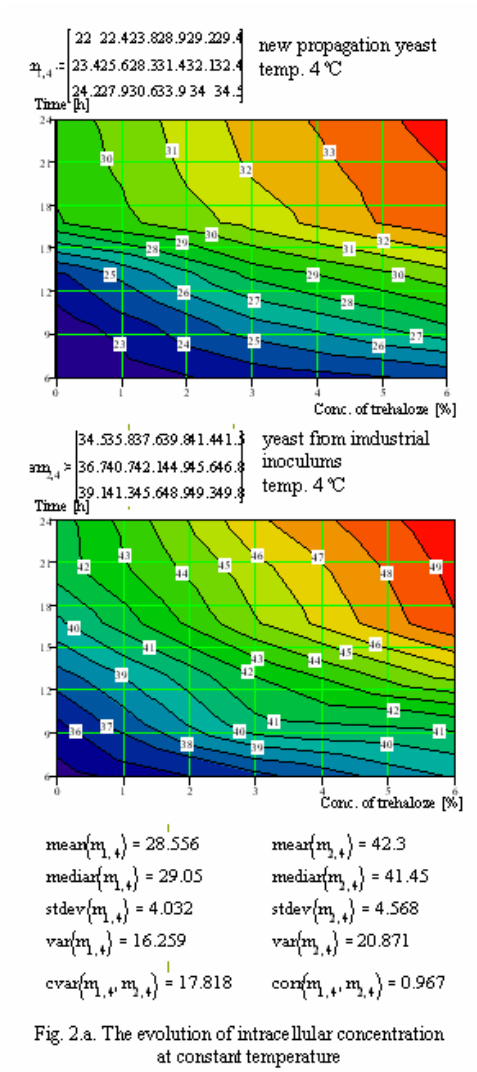

Fig. 2.a. The evolution of intracellular concentration at constant temperature

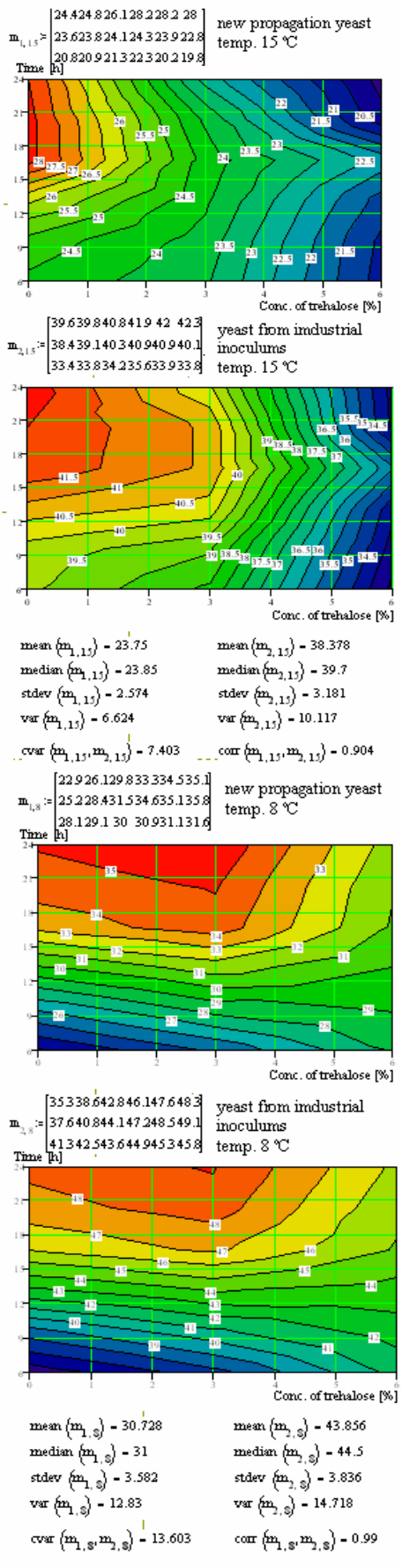

Fig. 2.b. The evolution of intracellular concentration at constant temperature

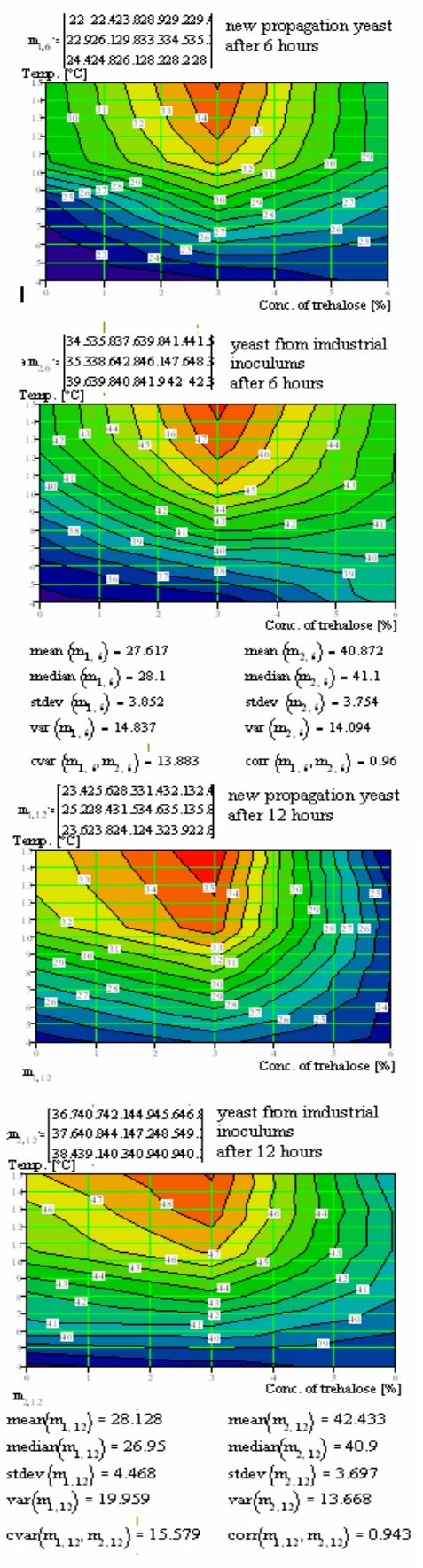

Fig. 3.a. The evolution of intracellular concentration at constant time interval

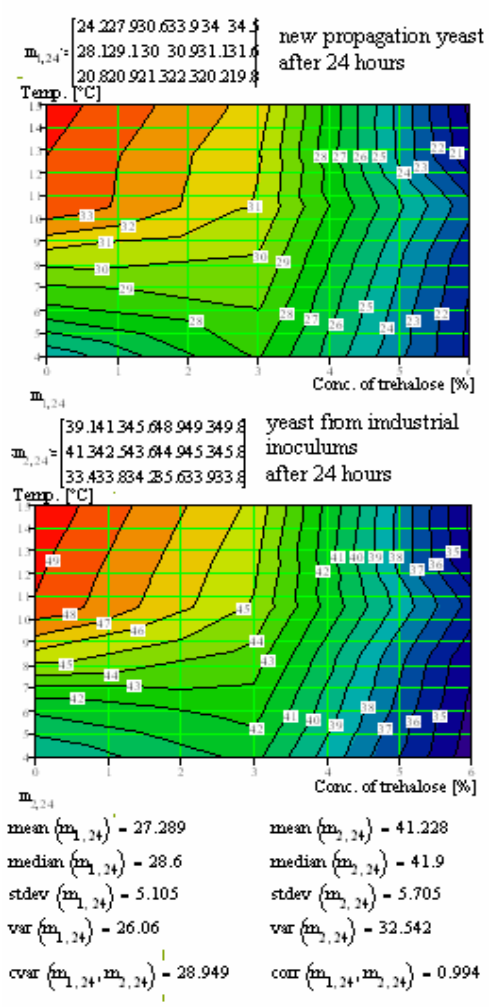

Fig. 3.b. The evolution of intracellular concentration at constant time interval

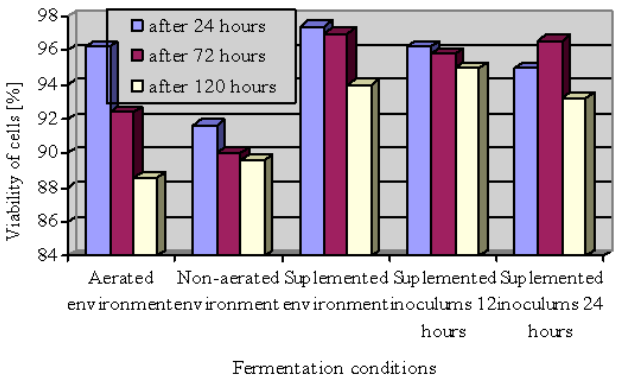

Fig. 4. Comparing of viability of cells from fermented environment in different conditions, after 24, 72, 120 hours

As is shown in figure 8.5, the viability of cells is the lowest in conditions offered by non-aerated medium. In the aerated medium, viability is initial low, but decreases against the end of the fermentation. The greatest number of viable cells was registered in the medium in which was linoleic acid, even under the form of a supplement for medium, or under the form of a supplement for inoculums yeast.

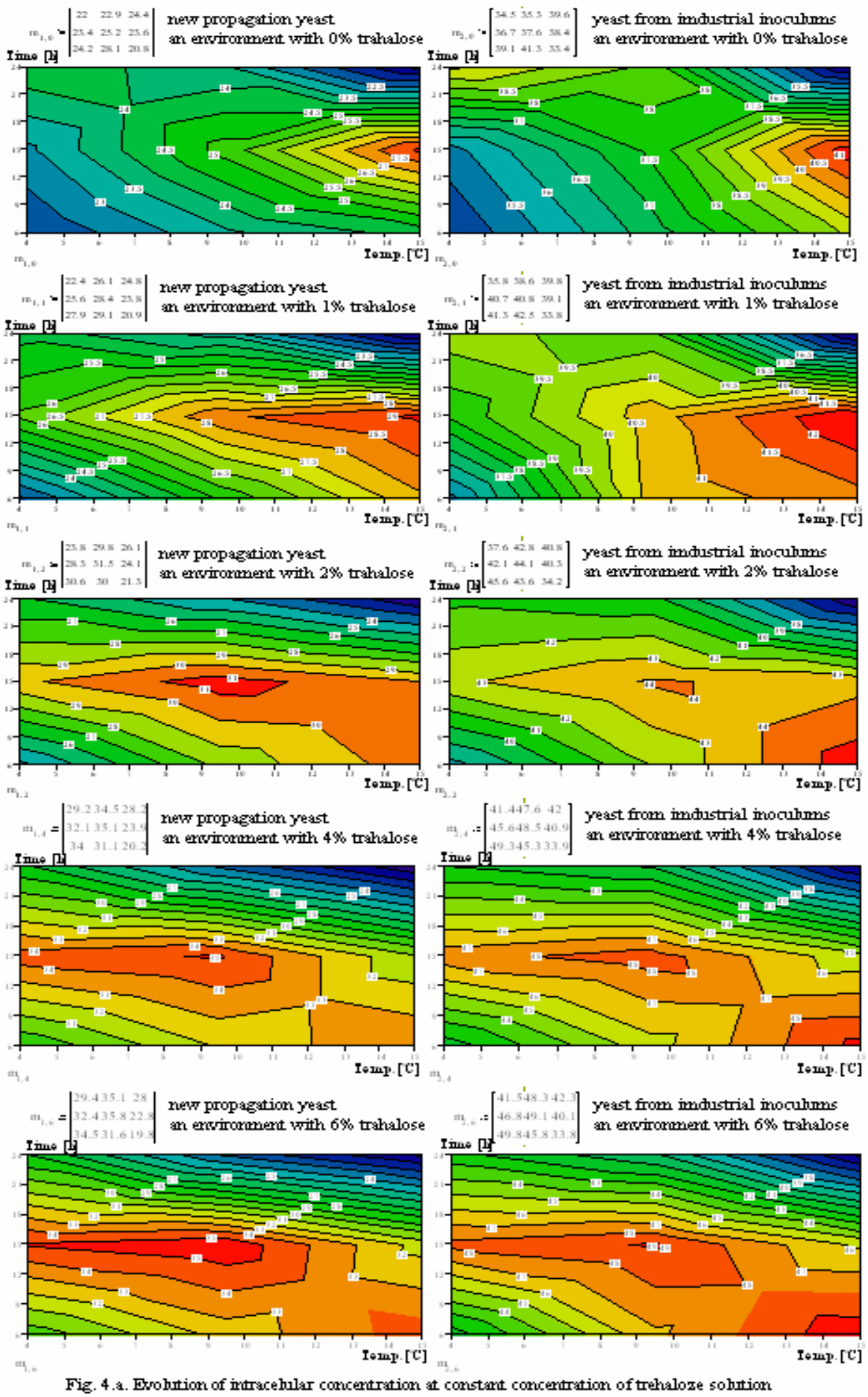

Fig. 4.a. Evolution of intracelular concentration at constant concentration of trehalose solution

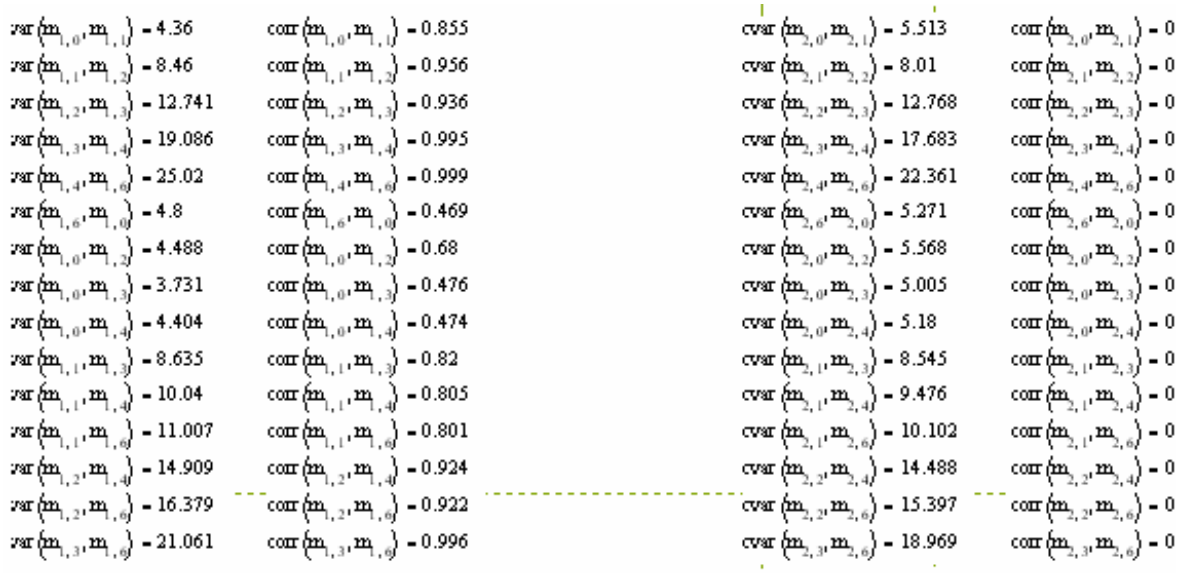

Fig. 4.b. Evolution of intracelular concentration at constant concentration of trehaloze solution

VI. Conclusion

After the progress recorded by the Pasteur's and Hansen's researches which obtained, through isolating techniques, the first culture of pure yeast, other ways to optimize the yeast fermentation activity was limited. This fact is due to that the classical techniques to improve biological the yeast stem (crossing and selection) are heavy, nonspecific and not always applicable to the non-spore beer yeast.

To improve the fermentation features of beer yeast stems, the most accesible direction seems to be that which uses the recombined ADN technique. The numerous examples of beer yeast stem transforming through recombined ADN technology demonstrate the potential of these techniques in case of yeast amelioration for substratums using (Lancashire, 1989), the degradation of β-glucons (Enari, 1987), the proteolize and the decresing of diacetyl level (Young and Hosford, 1987).

The technique of ADN recombination was used to counteract the problems arrised by level of diacetyl produced by some stems in quantities big enough. It is known that, formation of diacetyl and of 2-3 pentadioneies occures at primary fermentation by biosynthesis of valine and izoleucine. The yeasts doesn't produce the diacetyl, but the acetolactat precursor synthetized by action of acetohidroxiacid-syntetase on CoA acetyl and of piruvic acid came from glucidic katabolism.

Ethanol represents the stress factor by chemical type which appear in particular way at fermentation of concentrated beer worts when the concentration of accumulated ethanol arrives at values by 7-10% (vol.). The presence of ethanol determines the increasing of protons inflow due to the incresing of cell membrane permeability and due to accumulation of non-saturated greasy acides. The incresing of the ratio between non-saturated and saturated greasy acides implies the fluidization of cell membrane which is relationed with the elimination of ions and enzymes.

Moreover, researches on this direction and confirmed by obtained data through this study have associated the ethanol toxicity with losing of H+ATP-ase activity.

The modified test of combustion is a good indicator of viability, vitality and of one good enzymathic potential of yeast population which form the production inoculum used for sowing applicable especially on evaluation of yeast quality for repeated re-usings.

The obtained data after comparative study of yeast cells supplemented or not with linoleic acid that fermented in aerated, non-aerated or non-aerated but supplemented with linoleic acid mediums, indicate:

- The yeast contained in the supplemented inoculums demonstrated a growing and led to a fermentation degree comparable with that yeast which fermented the aerated non-fermented yeast.
- The cells from supplemented medium and those that belong to inoculums supplemented with linoleic acid with a contact time of 12, respectively 24 hours are characterized by a certain progressive dynamic regarding the fermentation intensity.
- The quantity of generated biomass is greater in the supplemented medium and in that fermented by cells from inoculums supplemented with linoleic acid.
- The greatest number of viable cells is recorded even in the medium in that was presented the linoleic acid, or in that fermented by cells supplemented with linoleic acid.
- The trehaloze intracellular content of the cells supplemented at a contact time of 12 hours, at the initial moment clear-cut superior, decrease drastically in the first 24 hours of fermentation, what indicates the increasing of the

cells' capability to synthesize the trehaloze, during the time of contact with the linoleic acid, to use as reserve-carbohydrate in lag stage, but also to remake the reserve after 120 hours of fermentation in a greater measure then the cells from aerated, non-aerated and supplemented with linoleic acid medium.

- The fermentation degree of the aerated non-fermented beers, or of those impregned with inoculum suplemented with linoleic acid is lower, but the differences are very small.
- The capability to reduce the diacetil, corelated with the beer's vitality, is clear-cut superior at the cells from the inoculum suplemented with linoleic acid.

The results indicate as alternative variant for medium aerating the variant of suplementing the beer inoculum with linoleic acid.

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