

# THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE KINETICS OF A BIOSYNTHETIC PROCESS

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## ABSTRACT

The efforts of specialists in the kinetics of biosynthetic processes are concentrated on the investigation of kinetic relations depending on a limiting stage of the process (diffusive or kinetic). If a kinetic study is needed, then the kinetics of the process in relation to the limiting component (substrate or a metabolic product) is investigated. The influence of environmental factors, whether thermodynamic (temperature, pressure) or parametric (pH and oxidation-reduction potential), is usually not considered. Investigations were carried out on the influence of temperature on the rate of formation and decay of an enzyme using the Arrhenius equation, on the influence of pH enzyme dissociation and on substrate rate of formation and decay in different stages of substance transformation into the cell material. These relations were generalized by a mathematical dependence of the growth rate on environmental temperature and pH value.

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## INTRODUCTION

Increased capacities of microbiological industries have resulted in a strong interest in the optimization of microorganism culture conditions. The whole set of parameters influencing the processes of microbiological synthesis can be divided into two groups.

The first group covers the substance concentrations used by microorganisms while the second one comprises the so-called physical as well as physicochemical factors: pH, eH, temperature, and pressure. Poorly-soluble substances injected by gas stream, e.g. oxygen, can also be included here, since their concentration in the medium not only depends on their partial pressure in the gas stream but also on physical and physicochemical factors which determine the solubility of gas components. This contribution deals with some parameters of the second group.

Much experimental data has been accumulated which characterizes the influence of these parameters on the growth rate and on different biosynthetic processes. Extremal character of this influence for the temperature, pH, eH values and, occasionally, for  $pO_2$  values is fairly well known. However, in

most cases a simple statement of facts or a purely formalistic quantitative description are more easily made. At the same time, it is quite evident that in order to ensure the optimum control which would react flexibly to different perturbations, it would be preferable to apply mathematical models having an adequate physical model as their basis.

The complexity of metabolic processes underlying the life of the cell has for long been an obstacle to attempts at constructing physical models of growth and biosynthesis. Therefore, the introduction and substantiation by Ierusalimskii<sup>1</sup> of the concept of 'bottleneck' has constituted a breakthrough in developing mathematical models of microbiological processes. It has become possible to apply the knowledge acquired on fermentation catalysis and on the mechanism of chemical kinetics to the construction of the models in question. One could also understand the frequent inadequacy of simple models and poor transfer of results of periodical process to continuous culture conditions.

Because the cell represents a complex system which breaks up into a number of subsystems, we can qualify each of the latter as a 'bottleneck' of its own. The model thus constructed will be sufficiently simple and at the same time flexible. We shall not dwell here on the principles of subdivision of the subsystems or on the description thereof. For our purposes it will suffice to single out two subsystems in the cell: (i) cellular membrane, and (ii) cytoplasm. One task will consist in defining the controlling subsystem and in describing the 'bottleneck'.

## EFFECT OF pH

### Influence of medium pH on intercellular pH

Let us consider the membrane of a cell as a semi-permeable system in the interior of which there is a colloid protein solution. Among twenty amino acids which construct the protein molecule there are dibasic acids (asparagine and glutamine) and diamino acids (lysine and arginine). It is quite natural that in the course of the formation of unbranched polypeptide threads, there remain free functional groups whose polar ends are oriented on the surface of the cellular membrane. Provided the protein amino-acid composition is known (cf. e.g. Refs. 2 and 3), one can estimate the 'concentration' of these functional groups and, accordingly, the intercellular pH of microorganisms. The part of these three-functional amino acids in a protein may total upwards of 25 per cent. Their average molecular weight is 100 to 150. If we suppose that the number of acidic and basic groups in the cellular cytoplasm is equal, then the content of functional groups will amount to 20 to 30 per cent, their molecular weight being 100 to 150, and the total 'concentration' of these groups is equal to 0.1 to 0.2M.

In this case, the intercellular pH of the cytoplasm will be equal to<sup>4</sup>

$$\text{pH}_c = 7 + \frac{1}{2}(\text{p}K_a - \text{p}K_b) \quad (1)$$

If the transport of an acidic product having the concentration  $[a]$  is in no way limited by the cell membrane, then as a result, its concentration in the

cell and in the medium will be equal. The pH value of the medium can then be obtained from

$$\text{pH}_m = \frac{1}{2}(\text{pH}_a - \ln [a]) \quad (2)$$

The increase of concentration  $[a]$  in the cytoplasm will result in a change of cellular pH, which, considering equations 1 and 2 can be calculated from the relation

$$[\text{H}^+] = \frac{[a]}{2} + \left( \frac{[a]^2}{4} + C^2 \frac{K_w}{K_a \cdot K_b} \right)^{\frac{1}{2}} \quad (3)$$

where

$$K_a = \frac{[\text{H}^+][a^-]}{[\text{H}^+a^-]}, \quad K_b = \frac{[\text{OH}^-][b^+]}{[b^+\text{OH}^-]}, \quad K_w = \frac{[\text{H}^+][\text{OH}^-]}{\text{H}_2\text{O}}$$

$K_a$ ,  $K_b$  and  $K_w$  are the constants of dissociation of the acidic, basic groups and of water, respectively.

Figure 1 presents the curves of the dependence of cell pH on the change of medium pH as estimated from equation 3 from which it follows that for

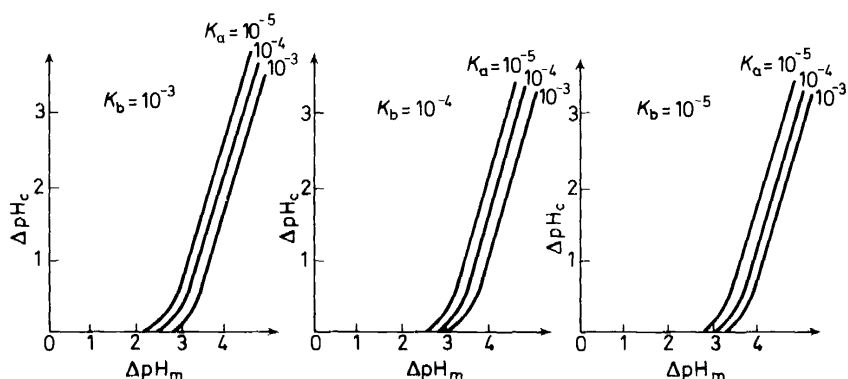


Figure 1, Dependence of intracellular pH on changes in pH of the medium

constant  $\text{p}K_a = 4$  and  $\text{p}K_b = 5$  (constants of dissociation of free functional groups: arginine  $-4.96$ , lysine  $-4.82$ , asparagine  $-3.86$  and glutamine  $-4.04$ ), the change of the absolute value of pH of the medium in the range 3.5 to 9.5 induces a pH change in the cell less than 0.03. In Figure 2 are indicated the estimated curves of growth rate and change of pH of cells of *Penicillium sp.* according to data cited from Ref. 5.

It is obvious that this estimation is idealized but the values of the results obtained will not change in any considerable way by assuming that the number of acidic group is not equal to that of the basic ones as their dissociation constants will also differ. According to our view, the above estimation removes the necessity of formulating a 'direct' impact of the pH medium on intercellular processes.

Thus, the pH manifests itself in a subsystem in which transport processes take place. This influence can express itself in different ways; either by the change of the degree of ionization of the substances necessary for cell synthesis (ions of different charge and molecules penetrate into the cell at different rates), or, by changing the membrane permeability. It is evident that the first assumption does not affect nonpolar or non-ionizing substances, e.g. glucose and oxygen. We shall therefore turn our attention to the second assumption.

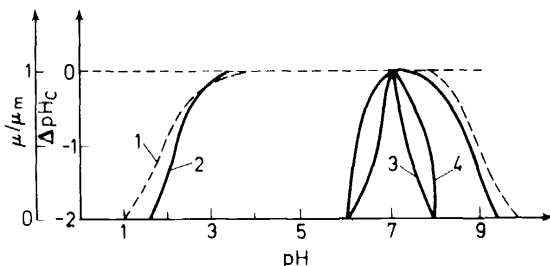


Figure 2. Dependences of the relative growth rate and intracellular pH on the change of pH of the medium for propagating *Penicillium sp.*: 1 - Experimental relative growth rate; 2 - Estimated curve of the change of intracellular pH with  $K_a = 10^{-4}$  and  $K_b = 10^{-5}$ ; 3 - Experimental curve of relative growth rate and the estimated value of the change of intracellular pH with  $K_a = K_b = 10^{-7}$ ; 4 - Curve of the change of the intracellular pH with  $K_a = K_b = 8 \times 10^{-7}$

### The influence of pH on the transport mechanisms of substances through biological membranes

Numerous studies were dedicated to the mechanism of transport through biological membranes. These can be divided into two groups. Recently, the view markedly prevailed, that this transport is an 'active' one<sup>6</sup>. According to this concept, the transport of substances through a membrane is carried out by means of special enzymes, 'permeases'. While interacting with a substrate molecule on the exterior surface of the membrane the 'permease' carries it to the interior surface from which the substrate molecule is removed by means of ATP. Consequently, the transport process itself is conjugated to the energy exchange processes. The main argument in favour of this theory is that in a number of cases the transport is carried out against a concentration gradient. This explanation is however not of a universal character.

Firstly, the transport direction is determined not by the concentration difference, but rather by the difference in thermodynamic potentials. This difference is found not only by the concentration ratio in different phases but also by the difference in standard thermodynamic potentials which can entirely attain a 'negative gradient'.

Secondly, normal diffusion through pores can ensure extraordinarily rapid cell saturation with nutrient substances.

Let us suppose that the glucose concentration in the medium is equal to  $10 \text{ g l}^{-1}$  and this acts upon a microbe cell with a diameter of  $1 \mu\text{m}$  and wall thickness  $100 \text{ \AA}$ , then the porous surface constitutes 1 per cent of the total cell

surface and glucose concentration in the cell is equal to zero. In this case, diffusion current towards the cell will be  $9.1 \times 10^{-14} \text{ mol s}^{-1}$ , and it follows from the Fick's law

$$C = \Delta C_0 \exp(-3D_m t/r),$$

$$t = \frac{1.53 r}{D_m} = \frac{1.53 \times 10^{-6} \times 10^{-4}}{5.2 \times 10^{-8}} \approx 3 \times 10^{-3} \text{ s}$$

where  $t$  is the time of the saturation of the cell. Accordingly, the passive transport mechanism cannot be neglected. When the transport is carried out by an enzyme, then as a result of an unfavourable pH change its activity may change and the 'bottleneck' may shift towards the membrane. This case is sufficiently well dealt with in the theory of fermentation catalysis<sup>7,8</sup>, and thus does not require any further elaboration. It should only be noted that the use of adequate equations to describe microbiological synthetic processes has yielded good results<sup>9</sup>. The influence of the pH value on the speed of 'passive' transport is of great interest.

According to the published data, it seems that there is practically always a surface charge on the cell surface<sup>10,11</sup>. The presence of the latter results in the formation of a double electric layer near the cell surface<sup>12</sup> obstructing diffusion through the surface. According to modern liquid theory<sup>13</sup>, diffusion in the liquid phase is considered to be a statistical transfer of molecules. Some activation energy is necessary for this transport and it is determined by the interaction forces between molecules. The presence of the surface charge of a cell increases the energy required for such transfer. Therefore, we can write

$$D_{av} = D_0 \exp(-a\varphi/RT) = D_0 \exp(-bf(\text{pH})/RT) \quad (4)$$

where  $a$ ,  $b$  are empirical coefficients and  $D_0$ ,  $D_{av}$  are diffusion coefficients.

Let us assume first that the density of surface charge is a constant value. According to data on x-ray structure analysis, the surface taken up by one length of a polypeptide chain in the form of a duplicated layer, constitutes  $30 \text{ \AA}^2$ . If the probability of finding a polar group in the period is equal to 0.1, then the density of a polar group will be  $3 \times 10^{13}$  of elementary charges per  $\text{cm}^2$  (Ref. 14). As follows from the theory of electrostatic charges of a sphere,  $\varphi = 4\pi q r$ . With  $r = 10^{-3} \text{ cm}$  and  $q = 3 \times 10^{13}$  elementary charges per  $\text{cm}^2$ , the potential in the field of charges will be  $\varphi = 6 \times 10^4 \text{ V cm}^{-1}$ . In this case, during ion shifting of  $5 \text{ \AA}$  the work spent will be  $3 \times 10^{-3} \text{ eV}$  or  $0.07 \text{ kcal}$ . This means that the surface charge of the cell cannot hinder diffusion of neutral molecules from the membrane surface into the cell.

However, the potential gradient in the pore can be higher, provided that pore channel walls are charged. The charge number on the pore wall can change due to diameter within the range from several dozens to several thousands. We can therefore consider the charge density to be about constant. Then, the potential in the middle of the pore will be equal to

$$\varphi = 4\pi\delta\rho \ln \{l - (1 + l^2)^{1/2}\} \quad (5)$$

where

$\rho$  is pore radius  
 $\delta$  is density of surface charge  
 $l$  is  $L/2\rho$   
 $L$  is pore length

At the pore entry the potential is

$$\varphi = 4\pi\delta\rho \ln \left[ \frac{\{2l^2 + 2(1 + l^2)^{1/2} + 1\}}{\{2l + (1 + l^2)^{1/2}\}} \right] \quad (6)$$

For the pore radius 50 Å and  $l = 1$  and wall thickness 100 Å the maximum difference of potentials with ion penetration of 5 Å (appropriate distance between cell centres), energy expenditure must be around 0.9 eV which corresponds to 20 kcal mol<sup>-1</sup>. In this case, the diffusion coefficient at  $T = 300$  K is decreased by 10<sup>14</sup> times, that is, molecular diffusion into the cell stops completely. Consequently, the presence of dissociating charged functional groups on the membrane walls allows the regulation of access of substrate molecules depending on pH value of the medium.

Charge on the cell surface is formed as a result of dissociation of basic and acidic groups which, as was stated above, are always present on the surface of protein globules. As is known, subject to the pH value of the medium, acidic and basic groups situated on the cell surface are dissociated in different manners. Denoting the dissociation constant  $K_a$  for acidic groups and the constant for the basic ones by  $K_b$ , then the fraction of dissociated acidic groups will be

$$\alpha_a = \frac{K_a}{K_a + [H^+]}$$

and of the basic ones

$$\alpha_b = \frac{[H^+]}{K_w/K_b + [H^+]}$$

In this case, the total density of the cell surface charge is

$$\delta = \alpha_b\varphi_b - \alpha_a\varphi_a = \frac{\varphi_b[H^+]^2 + K_a(\varphi_b - \varphi_a)[H^+] - \frac{K_w K_a}{K_b}\varphi_a}{[H^+]^2 - \left(\frac{K_w}{K_b} + K_a\right)[H^+] - \frac{K_w K_a}{K_b}} = 0 \quad (7)$$

where  $\delta$  is density of surface charge,

$\varphi_a, \varphi_b$  are density of acidic and basic groups respectively.

Let us introduce new variables

$$Z = [H^+](K_w K_a/K_b)^{-1/2}, \quad \alpha = (K_a K_b/K_w)^{1/2}, \quad \beta = \varphi_a/\varphi_b$$

then the density of the surface charge related to the density of the basic functional groups  $\varphi_b$  will be

$$\delta = \frac{Z^2 + \alpha(1 - \beta)Z - \beta}{Z^2 + (\alpha^2 + 1)Z/\alpha + 1} \quad (8)$$

Setting equation 8 equal to zero, it follows

$$Z = \alpha[(\beta - 1)/2 + \{(\beta - 1)^2/4 + \beta/\alpha^2\}^{1/2}] \quad (9)$$

This Z value can be considered optimal from the point of view of mass transfer. Substituting for Z,  $\beta$ , and  $\alpha$  values we obtain

$$[H^+]_{opt} = K_a \left[ \frac{\varphi_a - \varphi_b}{2\varphi_b} + \left\{ \frac{(\varphi_a - \varphi_b)^2}{4\varphi_b\delta^2} + \frac{K_w\varphi_b}{K_a \cdot K_b \cdot \varphi_b} \right\}^{1/2} \right] \quad (10)$$

or

$$pH_{opt} = pK_a + \log \left[ \frac{\varphi_a - \varphi_b}{2\varphi_b\delta} + \left\{ \frac{(\varphi_a - \varphi_b)^2}{4\varphi_b\delta^2} + \frac{K_w\varphi_b}{K_a \cdot K_b \cdot \varphi_b} \right\}^{1/2} \right] \quad (11)$$

If  $\varphi_a = \varphi_b$ , then

$$pH = pK_a + \log (K_w/K_a K_b)^{1/2} \quad (12)$$

Thus, the optimal pH value of the medium can be estimated provided the dissociation constants of the acidic and basic groups are known.

In Figure 3 curves are given of the dependence of density of surface charge on different pH values of the medium estimated by equation 8.

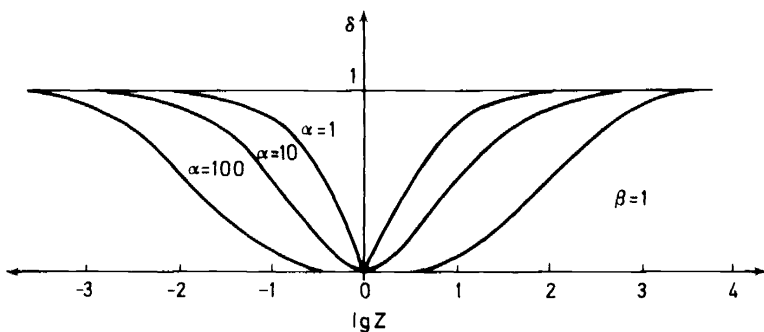


Figure 3. Dependence of the density of surface charge on the change of pH of the medium

The proposed mechanism of the pH influence explains well the existence of an interval of pH values within which pH does not affect growth speed<sup>5</sup>. As can be seen from Figure 3, the widths of this interval may differ. The optimal pH value may be also quite different. The diffusion mechanism explains well the narrowness of the limits of optimal pH zone which is not described in models on fermentation catalysis. In addition, in this case the transport is not conjugated with energy transfer. This is particularly essential while explaining the transport of O<sub>2</sub>. Since the ATP concentration increases with increasing O<sub>2</sub> concentration and vice versa, the transport is decreased, there exists a positive feedback system and the system examined is unstable. This contradicts the basic principles regulating living systems.

Rabotnova's recent data<sup>15</sup> indicate that the O<sub>2</sub> transport is restricted by the pH value because the transport speed of carbon source has not changed

beyond the optimal zone while the increased output of metabolic oxygen-containing products indicates the shortage of oxygen in cells.

While releasing low-molecular metabolites, the population of microorganisms increases the buffer capacity of the solution and thus restricts the changes in pH value. Since intercellular processes are mostly reversible, these metabolites may under certain circumstances be again absorbed by the population.

### A mathematical model taking account of the pH value of the medium

According to Fick's law, the diffusion rate of a substance into the cell taking account of the influence of the pH value of the medium can be represented in the following way

$$\frac{dS_1}{dt} = \frac{D_{av}}{\lambda} \pi r^2 \cdot \frac{3}{4\pi r^3} X(S - S_1) = \frac{D_{av}}{\lambda} \cdot \frac{3X}{4r} (S - S_1), \quad (13)$$

where

$$\begin{aligned} \pi r^2 & \text{ is surface of cell} \\ 4\pi r^3/3 & \text{ is volume of one cell} \\ D_{av}/\lambda & \text{ is coefficient of mass transfer} \\ S_1 & \text{ is substance concentration within the cell} \\ \lambda & \text{ is thickness of diffusion layer.} \end{aligned}$$

Starting from equation 13 we write a system of differential equations which describes the biosynthetic processes as follows

$$\begin{aligned} \frac{dX}{dt} &= \mu_m \frac{XS_1}{S_1 + K_S} \\ \frac{dS_1}{dt} &= \frac{3}{4} \frac{D_{av}}{\lambda r} X (S - S_1) - \alpha \mu_m \frac{XS_1}{S_1 + K_S} \end{aligned} \quad (14)$$

It follows from the stationary solution that

$$K_S S + \left( S - K_S - \frac{4\alpha\mu_m\lambda r}{3D_{av}} \right) S_1 - S_1^2 = 0 \quad (15)$$

hence

$$S_1 = \frac{A}{2} + \left( \frac{A^2}{4} + K_S S \right)^{1/2}, \quad \text{where } A = S - K_S - \frac{4\alpha\mu_m\lambda r}{3D_{av}}$$

or

$$A = S - K_S - \mu_m/K_0 \exp(-bf(\text{pH})/RT), \quad \text{where } K_0 = 3D_0/4\alpha\lambda r$$

Based on equations 14 and 15, assuming that the surface of pores is 1 per cent, their diameter is 50 Å, and wall thickness 100 Å,  $\text{p}K_a = 4$ ,  $\text{p}K_b = 5$ ,  $\mu_m = 0.3 \text{ h}^{-1}$ ,  $K_S = 1 \text{ g l}^{-1}$  for the culture *Candida tropicalis* curves were obtained for the change of maximal specific growth rate at



constant  $K_s$  and for variable  $K_s$  at constant  $\mu_m$ , depending on pH value (Figure 4).

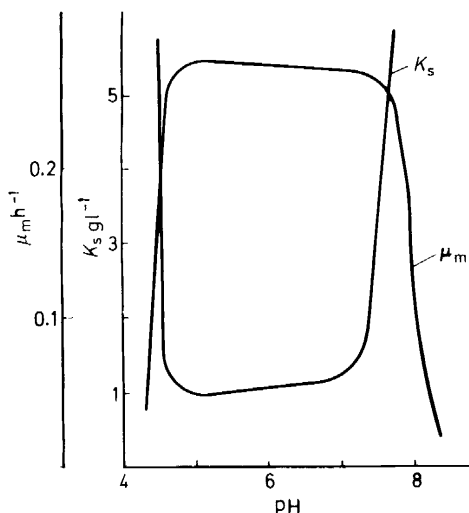


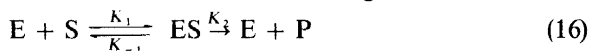
Figure 4. Dependence of the specific growth of *Candida tropicalis* microorganisms and the substrate constant on the change of pH of the medium

### EFFECT OF TEMPERATURE ON THE GROWTH RATE OF MICROORGANISMS

Since temperature affects the whole cell body and it exercises equal influence on all fermentation reactions, it is useful to consider its effect on the speed of the process in the 'bottleneck'.

#### Mathematical models

Suppose, the limiting link is described in the following scheme



where E is enzyme, S is substrate, P is product,  $K_1$ ,  $K_{-1}$ ,  $K_2$  are constants

As a result of a simple transformation we can derive from this the Monod equation

$$\frac{dX}{dt} = \mu_m \frac{SX}{S + K_s} \quad (17)$$

provided that

$$X = b_1 P, \quad E_0 = b_2 X,$$

where X is biomass concentration,  $E_0$  is total concentration of enzyme,  $b_1$ ,  $b_2$  are constants.

According to the Arrhenius law which is applicable within the range of a

narrow temperature interval, we assume the following form of dependence on temperature for the kinetic constants

$$K_1 = P_1 Z \exp(-E_1/RT), \quad K_{-1} = P_2 Z \exp(-E_2/RT), \\ K_2 = P_3 Z \exp(-E_3/RT) \quad (18)$$

where  $T$  is temperature in degrees K,  $R$  is the gas constant,  $Z$  is the number of collisions of reacting molecules,  $P_1, P_2, P_3$  are steric factors, and  $E_1, E_2, E_3$  are activation energies.

Thus we find that

$$K_S = a_1 \exp(\varepsilon_1/RT) + a_2 \exp(\varepsilon_2/RT); \quad \mu_m = a_s \exp(\varepsilon_3/RT) \quad (19)$$

$$\frac{dX}{dt} = a_3 \exp(\varepsilon_3/RT) \frac{SX}{S + a_1 \exp(\varepsilon_1/RT) + a_2 \exp(\varepsilon_2/RT)} \quad (20)$$

### Model verification by experiments

In order to check the adequacy of the models, batch and continuous experiments have been carried out.

The culture of *Candida tropicalis* was grown in shaking cultures in five parallel flasks on the Rieder medium (initial inoculation:  $0.5 \text{ gl}^{-1}$  at initial glucose concentration of  $10 \text{ gl}^{-1}$ ). Samples were taken every two hours to be analyzed for biomass and for the glucose content. Based on kinetic curves,  $K_S$  and  $\mu_m$  were obtained. Their dependence on cultivation temperature is given in *Table 1*.

*Table 1*, Experimental verification of equation 19

		Temperature, °C					
		25	28	30	35	38	40
$\mu_m$	Experiment	0.210	0.285	0.305	0.353	0.365	0.381
	Calculated	0.26	0.288	0.302	0.335	0.365	0.385
$K_S$	Experiment	5.03	3.27	2.12	1.51	1.92	2.87
	Calculated	5.5	3.04	2.16	1.53	1.86	2.87

Experimental data were treated according to equation 19. It was found that

$$K_S = 3.6 \times 10^{34} \exp(-49000/RT) + 3.8 \times 10^{-25} \exp(34300/RT) \\ \mu_m = 630 \exp(-4600/RT) \quad (21)$$

The calculated values are given in *Table 1*. Only at  $t = 25^\circ\text{C}$  the calculated  $K_S$  and  $\mu_m$  values differ noticeably from the experimental ones.

During continuous cultivation *Candida tropicalis* was grown in the Rieder medium with glucose concentration of  $10 \text{ gl}^{-1}$ . Air supply was  $11 \text{ l}^{-1} \text{ min}$ , r.p.m. = 2000, pH = 5.0, reaction zone volume was 1 litre, dilution rate  $0.15 \text{ h}^{-1}$ .

The biomass concentration was determined under steady state regime. Results obtained are given in *Table 2* below.

After treatment of experimental data, it was found that

$$\ln a_1 = 139.044, \ln a_2 = -33.224, \ln a_3 = 15.048, \varepsilon_1 = -115.02 \text{ kcal mole}^{-1}, \varepsilon_2 = 29.89 \text{ kcal mol}^{-1} \text{ and } \varepsilon_3 = -9.82 \text{ kcal mol}^{-1}. \quad (22)$$

The average standard error of the estimated values from experimental ones was  $\pm 0.21 \text{ g l}^{-1}$ , which confirms the reliability of the model. The multiplying correlation coefficient was equal to 0.96.

### Utilization of temperature as a control parameter in batch processes

It follows from equations 19 and 20 that increasing temperature decreases  $\mu_m$  but the growth rate increases, which is limited by a sharp increase of  $K_s$ . It is obvious that at high initial substrate concentrations the process may be conducted at higher temperatures without running the risk of an increased  $K_s$  value, but as the substrate is used up, the temperature should be decreased to that which corresponds to a certain optimum.

Utilizing the Pontryagin maximum principle<sup>16</sup> we can calculate the time change of temperature which would ensure optimal conditions of the process.

Let us first introduce the criterion of optimality  $Q$  in the form

$$Q = (X_1 - X_0)/\tau, \quad (23)$$

where  $X_1$  and  $X_0$  are the final and initial concentrations of the biomass,  $\tau$  is the duration of the process and  $X_0$  is usually given by technological considerations. At a fixed  $X_1$  value the problem is equivalent to that of a rapid action. Without referring here to the proof<sup>17</sup> let us state that from the maximum principle it follows that

$$\max_{\tau} H = \varphi \max_T f(X, T) \quad (24)$$

where  $H$  is the Pontryagin function and  $dX/dt = f(X, T)$ .

Thus, to build the optimal control, it is necessary to find for each  $X$  value the optimal  $T$  from the relation

$$\frac{\partial f(X, T)}{\partial T} = 0$$

Dividing the  $[X_0, X_1]$  interval into smaller parts and integrating within each interval at optimal  $T$ , we obtain the change in time and the process kinetic curve. The numerical solution of this task with  $K_s$  and  $\mu_m$  constant<sup>22</sup> resulted in almost a two-fold decrease of  $\tau$  compared with the process carried out at optimal constant temperature.

By following  $X_1$  from small values to greater ones and calculating  $\tau$  for each stage, we can easily determine the optimal conditions for carrying out the process.

### Utilization of temperature for optimal control of the continuous process

We have chosen the productivity of a continuous process  $Q_c$  as the criterion of optimality

$$Q_c = DX \quad (25)$$

where  $D$  is the dilution rate.

Under steady state conditions of continuous cultivation we can assume that near the stationary point, the 'bottleneck' does not shift along the metabolic chain, and this is why the Monod's equation may be used as a mathematical model.

Then the productivity is derived from the equation

$$Q_c = \alpha D \left( S_0 - \frac{K_s(T)D}{\mu_m(T) - D} \right), \quad (26)$$

where

$$\alpha = \frac{dS}{dX} = \text{const.}$$

Once  $D$  is fixed, we find the optimum as to  $T$  by solving the equation

$$dQ_c/dT = 0 \quad (27)$$

After substitutions and transformations equation 26 is reduced to

$$\begin{aligned} a_1 a_2 (\varepsilon_1 - \varepsilon_2 + \varepsilon_3) \exp(\varepsilon_2/RT) + a_1 a_3 \varepsilon_3 \exp(\varepsilon_3/RT) \\ = D a_2 (\varepsilon_1 - \varepsilon_2) \exp(\varepsilon_2 - \varepsilon_3/RT) + D \varepsilon_3 (\varepsilon_1 - \varepsilon_3) \end{aligned} \quad (28)$$

Solution of this equation gives the optimal temperature. It can be shown<sup>18</sup> that the solution always corresponds to the maximum provided that it exists in the physiological temperature range. It is evident that the value of the optimal temperature is affected by dilution rate. Accordingly, it is possible to find the optimum by two variables,  $D$  and  $T$ . At constant temperature, the dilution rate giving the maximal productivity is

$$D_c = \mu_m(T) \{1 - [K_s(T)/\{S + K_s(T)\}]^{1/2}\} \quad (29)$$

Substituting equation 9 into equation 25 and differentiating according to  $T$ , we obtain an equation for the optimal temperature

$$\begin{aligned} \varepsilon_2 a_2 \exp(\varepsilon_2/RT) + \varepsilon_1 a_1 \exp(\varepsilon_1/RT) \\ = \{a_1 \exp(\varepsilon_1/RT) + a_2 \exp(\varepsilon_2/RT)\} (a_1 \exp(\varepsilon_1/RT) \\ + a_2 \exp(\varepsilon_2/RT) + S_0)^{1/2} \end{aligned} \quad (30)$$

The dilution rate is calculated once  $T$  is found from equation 30.

The solution of equation 30 with the data presented above gave  $t_c = 33.8^\circ\text{C}$ ,  $D_c = 0.228 \text{ h}^{-1}$ . Here,  $Q_c = 0.84 \text{ kg m}^{-3} \text{ h}$  which is 25 per cent more than the maximum at  $D = 0.15 \text{ h}^{-1}$  (cf. Table 2).

Table 2. Steady state results of *Candida tropicalis* on glucose

		Temperature, °C							
		25	30	32	35	36	36.5	38	40
Productivity, $\text{gl}^{-1} \text{h}^{-1}$	experimental	0.41	0.65	0.71	0.66	0.62	0.61	0.60	0.42
	calculated	0.41	0.64	0.66	0.66	0.64	0.63	0.57	0.42
Biomass concentration, $\text{gl}^{-1}$	experimental	2.7	4.4	4.7	4.4	4.4	4.2	4.0	2.8
	calculated	2.7	4.3	4.5	4.4	4.3	4.3	3.9	2.9

The results obtained show that the temperature chosen as optimal at one dilution rate may not be optimal at another.

## EFFECT OF CONCENTRATION OF DISSOLVED OXYGEN ON THE VELOCITY OF MICROBIOLOGICAL SYNTHESIS

### Participation of oxygen in metabolic processes

At present, the view prevails that the transport of oxygen into cells is not limited, and therefore its concentration in the medium fully determines the velocity and direction of intercellular reactions. As was shown above, the correctness of this assumption is subject to serious doubts. We shall however consider a case where the transport is not limited, since the influence of the transfer rate will be always indirect.

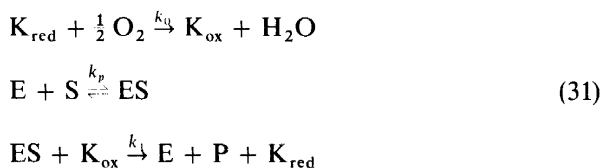
Available oxygen participates in two basic processes: (i) substrate oxidation and (ii) oxidative phosphorylation. The ATP resulting from phosphorylation is the main energy transmitter of the cell and the value of its concentration and ATP:ADP:AMP ratio is an essential indicator of the physiological state.

When glucose is used as substrate, then deficiency in ATP is responsible for the fact that AcCoA is not formed at an adequate rate and the process leads to alanine and lactic acid formation. The economy of this process is low so the expenditure coefficient ( $\alpha$ ) increases. On the contrary, an exceedingly high ATP:ADP ratio gives rise to the deceleration of amine-forming processes by some intermediate products of the Krebs cycle. The synthesis rate of amino acids, and, accordingly, that of biomass, is decreased. Thus, the oxygen concentration in the cell is an essential factor which determines both the rate of formation and the yield of desired products of microbiological synthesis. Oxygen concentration can also determine the composition of the biomass, as well as the lipid, protein and carbohydrate ratio within the cell.

### The mathematical model

Despite the fact that the qualitative explanation of the biochemical nature of the  $pO_2$  effect on the direction of a microbiological synthesis does not present any substantial difficulties, nevertheless, the quantitative description is a serious matter. We shall not deal with it in this paper.

Let us only examine in a simple way the  $pO_2$  effect on the growth rate. Assuming that the limiting link in the 'bottleneck' is the oxidation of a certain coenzyme with  $O_2$  participation and its interaction with the enzyme-substrate complex, we can write



where  $K_{\text{red}}$  is the reduced and  $K_{\text{ox}}$  is the oxidized form of coenzyme,  $k_0$ ,  $k_1$  are kinetic constants and  $k_p$  is the equilibrium constant.

From this scheme and utilizing the principle of the quasi-steady state, taking into account that

$$K_{\text{red}} + K_{\text{ox}} = K_0 = \text{constant}$$

we obtain, under usual assumptions, for the growth rate

$$\frac{dX}{dt} = \mu_m \frac{SXw}{K_w S + Sw + K_s w} \quad (32)$$

where  $w$  is concentration of the dissolved oxygen. Given high  $w$  values, oxygen does not influence the growth rate. At low rates, equation 32 assumes the shape of the Monod equation with the limiting influence of  $O_2$ <sup>19, 20</sup>.

The constants included in this equation are easily found if it is possible to carry out the process keeping  $w$  constant. Equation 32 is then reduced to

$$\mu = \mu_m^* \frac{S}{S + K_s^*} \quad (33)$$

where

$$\mu_m^* = \mu_m \frac{w}{K_w + w}, \quad K_s^* = K_s \frac{w}{K_w + w}$$

Having found  $\mu_m^*$  and  $K_s^*$  at different values of  $w$ , it is easy to find  $K_s$ ,  $\mu_m$ , and  $K_w$ .

In a more complicated case these constants can be found with the help of a computer if one has the curves of the time changes of  $X$ ,  $S$ , and  $w$ .

When building the model we should also include among other equations the oxygen balance

$$\begin{aligned} \frac{dX}{dt} &= \mu_m \frac{SXw}{K_w S + Sw + K_s w} \\ \frac{dS}{dt} &= -\alpha \\ \frac{dw}{dt} &= K_m(\gamma P_{O_2} - w) - \beta \frac{dX}{dt} \end{aligned} \quad (34)$$

where  $P_{O_2}$  is the partial pressure of  $O_2$  in the aerating gas,  $\gamma$  is the Henry constant,  $\beta$  is the  $O_2$  consumption per synthesis of unit weight of biomass,  $K_m$  is the volumetric coefficient of mass transfer of  $O_2$ .

The solution of this system at low  $K_m$  values gives rise to a characteristic rectilinear section in batch curves.

### THE INFLUENCE OF THE OXIDATION-REDUCTION SITUATION ON MICROBIOLOGICAL SYNTHESIS

Oxidation-reduction transformations play a leading part in processes of microbiological synthesis; in particular, the energy exchange in the cell represents a chain of conjugated oxidation and reduction processes. Oxidation and reduction transformations in the cell correlate with the oxidation-reduction situation of the surrounding medium.

It is quite understandable that the dependence between the biosynthesis proper (velocity, direction, etc.) and oxidation–reduction conditions of the medium, can be used as a control channel which is intimately linked with the physiological state of the population of microorganisms by the activity of the latter. An important advantage of such a channel is the simplicity of instrumental set-up for the whole control design.

Despite a considerable volume of publications proving the intense interest of researchers in this problem, they lack generalizations, are mostly of descriptive character, are not comparable and are sometimes contradictory. As for electrometrical methods for the study of oxidation–reduction processes, as applied to microbiological media, these already have a 5-years' history.

Analysing the reasons for this situation we can single out three principal reasons :

(i) The culture medium has a complex chemical composition. Qualitative and quantitative characteristics of oxidation–reduction systems in solutions are not existent. Oxidation–reduction systems themselves are fairly weak in terms of reactivity and are present in the medium in fairly low concentrations. It is natural that under these conditions competing oxidation–reduction systems, and, in particular, the oxygen ones, play an essential part. By their nature, the processes of cultivation of microorganisms are not equilibrium ones. Oxidative and reductive properties depend considerably on kinetic factors. This gives rise to the thermodynamical uncertainty of the oxidation (or reduction) capacity of the medium.

(ii) The medium properties mentioned alert researchers as far as the reliability of the data obtained is concerned. Actually, different types of probe electrodes behave differently under similar conditions. The basis for standardization of definitions is not complete. This brings about incompatible and frequently contradictory results of studies.

(iii) The selection of oxidation–reduction systems to effect the oxidation–reduction situation as desired, represents an independent and rather complicated problem.

It is quite clear that even without thoroughly examining and analysing the reasons for such an unsatisfactory situation in terms of possibilities of the study of the oxidation–reduction situation of the medium inhabited by microorganisms, without research into special methodological and measurement techniques based on fundamental instrumental technique for measurement of redox potentials, reliable research studies on the elucidation of relationships between oxidation–reduction situation and the life of microorganisms and, thus, on the elucidation of the parameters of this important channel of control of the processes of biosynthesis can be initiated (conceived).

On the basis of (a) theoretical assumptions on the interactions in a microbiological medium, characterised by poor buffer properties in terms of oxidation–reduction reactions, and by competing oxygen systems present practically in all microbiological media ; (b) indicator redox electrode as well as engineering methods for the evaluation of performance and degree of individual reversibility of redox electrodes for different oxidation–reduction systems, and (c) actual experimental evidence on model and real micro-

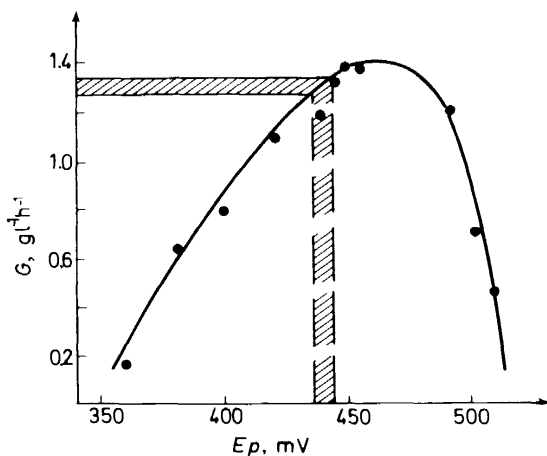
biological media obtained with the help of a special multielectrode apparatus, we have reached the following conclusions.

(i) Under conditions of poor buffer capacity the oxidation potential loses its definite thermodynamic meaning to become a steady state potential dependent on specific individual properties and on the type of electrode. The steady state potential has its instrumental meaning and characterizes in a way the oxidation-reduction properties of the surrounding medium, and their variations during cultivation.

(ii) The estimation of the results of definitions with metal electrodes as indicating redox electrodes under conditions of poor buffering properties of the medium and in the presence of oxygen can be made, provided the reversibility of metal electrodes to oxygen as a competing potential-determining system is taken into account.

(iii) The glass redox electrode is the best one for these purposes; its information is practically independent of the oxygen content in the medium.

Taking the above into consideration, special investigation has been made to reveal the relationship between technological parameters of the culture medium and the activity of the biosynthetic process. The dependence of productivity on the value of the electrode potential of a glass redox electrode is shown in *Figure 5*. The zone of potential values and the corresponding



*Figure 5.* Dependence of the process productivity on the value of electrode potential

zone of productivity characteristic for the oxidation-reduction situation created by proper metabolic activity of yeast *Candida utilis* (without reagent being added) is shaded.

The dependence is extremal; the process may be optimized using this parameter. Under the optimal regime the productivity increases by 10 to 15 per cent.

A mathematical model of the cultivation process which would take into account oxidation-reduction conditions of the medium is not yet conclusively formulated.



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