

Short communication

Study of influence of air ions on the growth rate of yeast *Saccharomyces Cerevisiae* based on analytical growth model

ABSTRACT

The influence of the concentration of ions in a nutrient medium both on the surface charge and potential of a yeast cell and diffusion of nutrients through its membrane and on the productivity of baking yeast was studied theoretically. It was shown that the ionized molecules of air components (first of all, of oxygen and nitrogen) changes the concentration of ions in the medium, the surface charge and potential of the yeast cell. This feature affects the diffusion of nutrients through membrane into the cell. The growth rate of the yeast mass depends on the amount of electricity, transported by air ions in the nutrient medium, and is limited by its certain value. That is why the yeast mass growth equation take into account the amount of electricity transported by air ions into the nutrient medium. A priori calculation shows that the productivity of the baker's yeast can increase by 12...17%.

Keywords: yeast cell; electricity; ion; potential; diffusion; growth kinetics.

1. INTRODUCTION

Saccharomyces cerevisiae, known as brewer's yeast or bread yeast, is important for some biotechnological applications [1]. The yeasts have received considerable attention because of the potentiality of cells. They are applied as starter in fermented beverage industries, as probiotic yeast, etc. The yeasts are also used to produce cellular components (proteins, polysaccharides) which are as functional ingredients in the food industry [2, 3]. In this regard, the task is to obtain large amounts of yeast mass rather than ethanol, as happens in other applications [3].

It is known that the yeast growth rate depends on a lot of factors (the oxygen concentration in the medium, the nutrient concentration, the nutrient solution composition, the medium temperature, and the diffusion of nutrient ions through the pores of the cell membrane, etc.) [3]. For example, *Saccharomyces cerevisiae* must grow in a medium with available oxygen and low initial sugar concentration [4, 5]. The problem of baker's yeast production is low cell productivity and incomplete use of the nutrient medium potential. This problem can be solved by thermal, chemical, mechanical, electrophysical and other methods [6-12]. The most promising for development are electrophysical methods, one of which is the treatment of a nutrient medium with yeast using air ions.

The use of electromagnetic fields for biofuels and bioenergy applications and the underlying possible mechanisms extensive discussed in [7]. The latter indicates that the low-frequency electric fields effects at enable ionic redistribution as well as a higher potential difference in the cellular suspension. It is also hypothesized that the pulsed electric fields stimulate cytoplasmic membrane modifications on account of temporary electroporation [13]. In [14] it

is also indicated that external electric fields improve nutrient transfer through the modified cell membrane via pore formation.

The effect of negatively charged oxygen and nitrogen ions in the air on the nutrient medium of yeast *Saccharomyces cerevisiae* is studied [4, 5, 7, 15]. It is known that hydrogen peroxide is the main active factor of air ions on the nutrient medium of yeast [16]. This factor changes the concentration of ions in the nutrient medium, activates the formation of new substances and the growth of yeast cells [17]. Changes in the surface charge and potential of a living cell affect the diffusion of nutrients through its membrane and, consequently, its growth [18, 19].

The objective of the present study was to establish theoretically the influence of the concentration of ions in a nutrient medium both on the surface charge and potential of a yeast cell and on the diffusion of nutrients through its membrane.

2. MATERIALS AND METHODS

Kinetic models are a tool to study the yeast behavior, metabolism, growth. There are a number of the structured and unstructured models for description of microbial cell growth [20-23]. Usually, the unstructured models describe cell growth kinetics and can be used to describe this process under various operating conditions (temperature, pH, and other adjustable parameters). Compared to the unstructured kinetic models, structured models are usually complex to estimation of kinetic parameters, mainly because of nonlinearities, the large number of parameters. Here we will consider influence of air ions on the growth of yeast *Saccharomyces cerevisiae* based on the model adapted from [24-31].

2.1. CONCENTRATION OF CHARGED PARTICLES IN THE NUTRIENT MEDIUM, CELL SURFACE CHARGE AND POTENTIAL

The change in the molar volume concentration of substance (mol/m^3) for an infinitely small time $d\tau$ in a medium volume where there occurs a current generated by the flow of air ions is:

$$\Delta C_{k,a_j} = \frac{1}{VF} \int_0^\tau I n_{k,a_j} d\tau, \quad (1)$$

here V is the volume of a processed medium, m^3 ; I is the instantaneous strength of the current flowing through the medium for a time $d\tau$, A; $F = 96485$ C/mol is Faraday's number; n_{k,a_j} is the instantaneous number of transfer of the k th cation or the j th anion for a time $d\tau$.

Transform (1) using the amount of electricity:

$$\Delta C_{k,a_j} = \frac{1}{F} \int_0^{Q_V} n_{k,a_j} dQ_V, \quad (2)$$

here $dQ_V = Id\tau/V$ is the amount of electricity transported by the air ions flowing through the medium, C/m^3 .

Allowing for Faraday's law, the molar volume concentration of negative ions C_{f^-} (mol/m^3) is obtained as a result of air ion processing of the medium:

$$C_{f^-} = C_{0f^-} + \frac{Q_V}{F} \eta_{f^-}, \quad (3)$$

here C_{0i^-} is the initial molar volume concentration of negative ions, mol/m³, and η_{i^-} is the current output of negatively charged particles.

The total yeast cell surface density ρ_s (C/m²), generated, for example, by the cations of kind i^+ [19], is:

$$\rho_s = \frac{\rho_b C_{i^-}^2 + K_a(\rho_b - \rho_a)C_{i^-} - \rho_a \frac{K_a K_w}{K_b}}{C_{i^-}^2 + 2K_a C_{i^-} + \frac{K_a K_w}{K_b}}, \quad (4)$$

where ρ_a , ρ_b is the surface density of the charge of acid and basic groups, respectively, C/m²; K_a , K_b , K_w are the dissociation constants of acid/basic groups and water, respectively, mol/m³.

At the same time,

$$K_a = \frac{C_{H^+} C_{i^-}}{C_{H^+ i^-}}; \quad K_b = \frac{C_{OH^-} C_{i^+}}{C_{OH^- i^+}}; \quad K_w = \frac{C_{H^+} C_{OH^-}}{C_{H_2O}}, \quad (5)$$

C_{i^+} is the concentration of positively charged ions, mol/m³; C_{H^+} , C_{OH^-} is the concentration of ions of hydrogen and hydroxide, respectively, mol/m³; $C_{H^+ i^-}$, $C_{OH^- i^+}$, C_{H_2O} is the concentration of acid/basic groups and water, respectively, mol/m³.

Assume that the yeast cell is shaped as a sphere covered with negative-sign ions with a surface density ρ_s . The potential of the sphere surface is governed by

$$\varphi_s = \frac{1}{4\pi\epsilon_0\epsilon_s} \int \frac{\rho_s}{R_c} dS. \quad (6)$$

where $\epsilon_0 = 8.85 \cdot 10^{-12}$ F/m is the electric constant; ϵ is the relative dielectric constant of the nutrient medium; S is the cell surface area, m²; $R_c = 5 \cdot 10^{-6}$ m is the cell radius [24].

Thus, the current flowing in the form of air ions changes the concentration of charged particles in the nutrient medium (3), cell surface charge (4) and potential (6) that in turn determine the diffusion rate and direction of substances through the cell membrane.

2.2. DIFFUSION OF IONS INSIDE THE CELL

The concentration of ions within a living cell can be ten times different from their concentration in the ambient medium, the concentration gradients of different-sign ions being observed. This is attributed to transport dynamics of ions through the membrane and depends on the properties of a specific cell. The concentration difference of ions outside and inside the cell under normal conditions gives rise to voltage between cytoplasm and ambient nutrient medium. It ranges from 50 to 70 mV [25, 26]. When air ions are applied, this potential difference can be larger than the membrane voltage breakdown (100...200 mV) [27].

Calculate the concentration gradient of ions at the cell edge, during the creation of which there arises a potential difference enough for membrane breakdown.

The diffusion equation for ions inside the cell pore is of the form [28]

$$\frac{d^2C}{dr^2} + \frac{2dC}{rdr} = 0 \quad (7)$$

at the boundary conditions: $r = R_c$, m ; $C = C_1$ is the concentration of ions at the cell surface, mol/m^3 ; $r = \infty$, $C = C_0$ is the concentration of ions in a region far from the cell surface, mol/m^3 ; here $C(r)$ is the concentration of ions at a distance r from the center of the cell, mol/m^3 .

The solution to (7) assumes the form:

$$C(r) = C_0 + \frac{(C_1 - C_0)R_c}{r}. \quad (8)$$

The diffusion flow of ions Φ (mol/s) through the surface of the cell with allowance for its spherical symmetry is:

$$d\Phi = -D_0C(r)dS, \quad (9)$$

or

$$\Phi_0 = -D_0 \int C dS = -4\pi R_c^2 D_0 \frac{dC}{dr}, \quad (10)$$

where $D_0 \approx 10^{-9} \text{ m}^2/\text{s}$ is the diffusion coefficient of substance in the medium [19]; dS is the surface element of the cell, m^2 .

From (10), one obtains

$$\frac{dC}{dr} = -\frac{(C_1 - C_0)R_c}{r^2}, \quad (11)$$

at $r = R_c$

$$\frac{dC}{dr} = -\frac{C_1 - C_0}{R_c}, \quad (12)$$

i.e.

$$\Phi = 4\pi R_c D_0 (C_1 - C_0). \quad (13)$$

On the other hand-side, by definition

$$\Phi = C_1 \vartheta S = 4\pi R_c^2 C_1 \vartheta, \quad (14)$$

where ϑ is the average ion velocity, m/s .

Taking into account (6) and (12) yields the average ion velocity in a pore:

$$\vartheta = \frac{C_1 - C_0}{C_1} \frac{D_0}{R_c}, \quad (15)$$

or

$$\frac{\vartheta}{C_1} = \frac{C}{C_1} \frac{D_0}{R_c}, \quad (16)$$

where C/C_1 is the concentration gradient of ions on the cell membrane.

In turn, the drift velocity of an ion is associated with the potential difference φ (V):

$$\vartheta_d \approx \lambda \frac{\varphi}{l}, \quad (17)$$

where λ is the ion mobility in the medium, $m^2/(s \cdot V)$; l is the distance between different-potential regions, m.

Assume that $l = 1.5\delta$, where δ is the membrane thickness, m.

From (16) and (17), one obtains

$$\frac{C}{C_1} \frac{D_0}{R_C} = \lambda \frac{\varphi}{l}, \quad (18)$$

$$\varphi = \frac{C}{C_1} \frac{D_0 l}{R_C \lambda}. \quad (19)$$

Relation (19) permits calculating the concentration gradient of ions outside and inside the cell. It provides a required potential difference φ .

Bearing in mind the known gradient C/C_1 , relation (19) may be used to calculate a resulting potential difference. In this case, the concentration gradient can be directed to both sides, i.e., varying the ion composition of the medium makes it possible to change electric charge and cell behavior.

Relation (19) has been obtained while assuming the transport of one-kind ions only through the membrane. In the general case, relation (19) takes the form:

$$\varphi = \frac{D_0 l}{R_C} \left[\frac{C_{(1)}}{\lambda_1 C_1} + \frac{C_{(2)}}{\lambda_2 C_1} + \dots + \frac{C_{(i)}}{\lambda_{(i)} C_1} \right], \quad (20)$$

where $C_{(i)}/C_1$ is the gradient of i th kind-ions that participate in transport through the membrane.

Assume that the potential breakdown difference for the membrane $\varphi_{bd} = 150$ mV. Then based on (20), the concentration gradient of corresponding ions is obtained:

$$H^+ : C/C_1 = 150; \quad NH_4^+ : C/C_1 = 200; \quad K^+ : C/C_1 = 250; \quad Na^+ : C/C_1 = 130; \quad OH^- : C/C_1 = 810; \\ NO_3^- : C/C_1 = 250; \quad O_2^- : C/C_1 = 3700.$$

Using Nernst's equation

$$\varphi = \frac{D_0 l}{R_C} \left[\frac{C_{(1)}}{\lambda_1 C_1} + \frac{C_{(2)}}{\lambda_2 C_1} + \dots + \frac{C_{(i)}}{\lambda_{(i)} C_1} \right], \quad (21)$$

$\varphi = 144.6$ mV, i.e., the results are practically identical. Here R is the universal gas constant, J/(mol·K); T is the absolute temperature, K, at a total concentration gradient with regard to the ion mobility $C/C_1 = 250$.

It can then be assumed that the diffusion of ions, providing a normal function of a cell, depends on the electric field generated by the charge of ions around the cell and in the cell itself.

2.3. DIFFUSION RATE OF SUBSTANCE INSIDE THE CELL

According to Fick's law, the diffusion rate of substance inside the cell is:

$$\begin{aligned} \frac{dM_C}{d\tau} &= \frac{D_\phi}{h} \pi R_C^2 \frac{3M}{4\pi R_C^3} (M_S - M_C) = \\ &= \frac{3 D_\phi M}{4 h R_C} (M_S - M_C), \end{aligned} \quad (22)$$

where τ is the diffusion time, s; D_ϕ is the coefficient of diffusion of ions into the cell through the membrane pore, m^2/s ; M is the specific volume of cells in the medium, m^3/m^3 ; h is the diffusion layer thickness, m; M_C is the substance concentration in the cell, kg/m^3 ; M_S is the substance concentration in the medium, kg/m^3 ; πR_C^2 is the cell surface area, m^2 ; $\frac{4}{3} \pi R_C^3$ is the volume of one cell, m^3 .

Diffusion of ions into the cell through the membrane pore in the presence of the cell surface potential, m^2/s , [29] is:

$$D_\phi = D_0 \exp\left(-\frac{nF|\varphi_S|}{RT}\right), \quad (23)$$

where n is the empirical coefficient and φ_S is the cell surface potential, V.

Assume that the cell membrane pore represents a tube with a radius R_P and a length h (Fig. 1). Upon transformations, with the consideration of pore sizes formula (6) takes the form:

$$\varphi_S = \frac{\rho_S R_P}{2\varepsilon_0 \varepsilon} \int_0^h \frac{dx}{\sqrt{R_P^2 + x^2}}, \quad (24)$$

where x is the distance along the tube axis from the pore entrance to the section plane passing through the middle of a strip dS , m, pictured in Fig. 1 and $R_P = 2.5 \cdot 10^{-9}$ m is the cell pore radius [24].

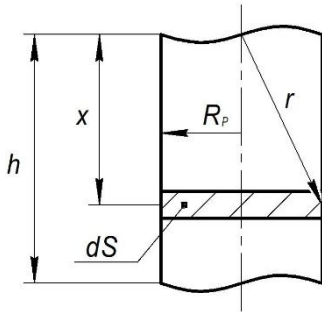


Fig. 1. Potential calculation inside the membrane pore

Solution to (24) can be written as:

$$\varphi_S = \frac{\rho_S R_P}{2\varepsilon_0 \varepsilon} \ln \left(\frac{x + \sqrt{R_P^2 + x^2}}{R_P} \right) \Bigg|_0^h, \quad (25)$$

From (25), the potential at the pore entrance is:

$$\varphi_s = \frac{\rho_s R_p}{2\varepsilon_0 \varepsilon} \ln \left(\frac{h + \sqrt{R_p^2 + h^2}}{R_p} \right), \quad (26)$$

2.4. YEAST MASS GROWTH

With (22) taken into account, according to Michaelis–Menten's equation, biosynthesis can be described by the system of differential equations [30, 31]:

$$\begin{cases} \frac{dM}{d\tau} = \mu \frac{M \cdot M_C}{M_C + K_S}, \\ \frac{dM_C}{d\tau} = \frac{3 D_\phi M}{4 h R_C} (M_S - M_C) - \alpha \mu \cdot \frac{M \cdot M_C}{M_C + K_S}, \end{cases} \quad (27)$$

where K_S is the substrate constant, kg/m³; α is the chemical activity the medium and μ is the yeast specific growth rate, s⁻¹.

Based on the time-independent solution to (27), one has:

$$(M_S - M_C)(M_C + K_S) - \frac{4\alpha\mu h R_C M_C}{3D_\phi} = 0,$$

$$K_S M_S + \left(M_S - K_S - \frac{4\alpha\mu h R_C}{3D_\phi} \right) M_C - M_C^2 = 0.$$

Upon solution, the substance mass in the cell is

$$M_C = \frac{A}{2} + \sqrt{\frac{A^2}{4} + K_S M_S}, \quad (28)$$

where the factor

$$A = M_S - K_S - \frac{4\alpha\mu h R_C}{3D_\phi} = M_S - K_S - \frac{\mu}{K_0 \cdot \exp\left(-\frac{nF|\varphi_s|}{RT}\right)} \quad (29)$$

where $K_0 = \frac{3D_0}{4\alpha h R_C}$.

Substituting (29) into the first equation of system (28) yields the yeast growth rate equation:

$$\frac{dM}{d\tau} = \mu \frac{M_C \left(\frac{A}{2} + \sqrt{\frac{A^2}{4} + K_S M_S} \right)}{K_S + \frac{A}{2} + \sqrt{\frac{A^2}{4} + K_S M_S}}, \quad (30)$$

or

$$\Delta M = \int_0^\tau \mu \frac{M_{C0} \left(\frac{A}{2} + \sqrt{\frac{A^2}{4} + K_S M_S} \right)}{K_S + \frac{A}{2} + \sqrt{\frac{A^2}{4} + K_S M_S}} d\tau, \quad (31)$$

Integrating (31) within given ranges provides the equation for the biomass specific growth under the influence of air ion processing:

$$\Delta M = \mu M_{co} \tau \left[\frac{A}{2} + \sqrt{\frac{A^2}{4} + K_s M_s} - K_s \ln \left(K_s + \frac{A}{2} + \sqrt{\frac{A^2}{4} + K_s M_s} \right) \right] \quad (32)$$

Equation (32) confirms that the yeast biomass growth depends on the cell surface potential φ_s that determines the diffusion of ions into the cell (29). Mathematically, it describes the change in the productivity of the baker's yeast and the medium when acted upon by air ions.

3. YEAST MASS GROWTH RESULTS

The proposed analytical model allows computing: the concentration of negative ions with the consideration of the amount of electricity flowing through the medium when (3) undergoes transformation; the total density of the surface charge of the yeast cell (4); the potential at the cell pore entrance (26); the simplification factor A (29); the yeast biomass growth (32) per m^3 of the nutrient medium. The results are depicted in Fig. 2.

Fig. 2 illustrates that the yeast biomass can increase per 15.5 kg/m^3 of the nutrient medium when processed by the amount of electricity ranging from 375 to 425 C/m^3 (m^3 of the nutrient medium contains $90 \dots 110 \text{ kg}$ yeast).

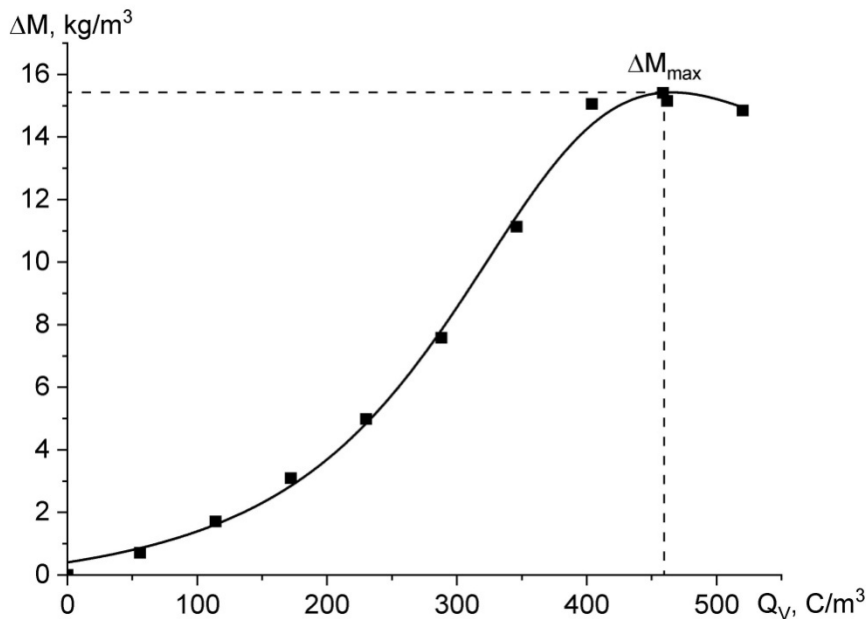


Fig.2. Change in the yeast biomass growth when acted upon by air ions according to (31)

4. CONCLUSION

The yeast *Saccharomyces cerevisiae* utilized in this study were able to growth with a high yield and productivity under applied electric field conditions. The electric field effects at

enable ionic redistribution as well as a potential difference on the cell. It is also supposed that electric field improve nutrient transfer through the cell membrane via pore formation.

Solution of the equations used to model the yeast growth kinetics established that the yeast mass production was associated with following mechanisms. Introducing the ionized air (first of all, oxygen and nitrogen) changes the concentration of ions in the medium. The ions affect the surface charge and potential of the yeast cell. This in turn affects the diffusion of nutrients through membrane into the cell. The yeast mass growth rate depends on the amount of electricity, transported by air ions into the nutrient medium, and is limited by its certain value. So, the yeast biomass growth equation depending on the amount of electricity transported by air ions in the nutrient medium too. A priori calculation shows that the productivity of the baker's yeast can increase by 12 to 17% for the conditions used in this study.

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