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IMPACT OF THE CELL WALL ON CYANIDE BIODEGRADATION IN A MODEL OF RESPIRATORY MECHANISM

UDC 539

Based on the general model of the respiratory mechanism of cyanide degradation by microorganisms, we introduce the impact of the cell wall on the degradation process under the conditions with and without the initial short-term pulsed electric field treatment. The research is conducted using non-linear phenomenological equations, and the solution approximation is obtained. Theoretical and experimental data are compared, and they are in good agreement. We demonstrate that the initial short-term pulsed electric field treatment increases the permeability of cyanide through the cell wall, as well as the rate of activation of the respiratory chains. The steady-state solutions and the maximum rate of cyanide addition are derived under the conditions that cyanide is continuously added to the solution with bacteria, and there is no initial pulsed electric field treatment.

K e y w o r d s: cell wall, cyanide, pulsed electric field, respiratory chain, porin, permeability, passive diffusion.

1. Introduction

The role of a cell wall in the metabolic processes of unicellular organisms in aggressive environments is not sufficiently elucidated. In addition to the obvious protective functions, the cell wall performs certain regulatory functions. But how this happens in each specific case is currently not sufficiently studied. In the case of the impact of cyanide on the metabolic activity of bacteria, it is obvious that the concentration of cyanide in the region of the cell wall near the cytoplasmic membrane has a decisive role due to the fact that cyanide acts precisely on the respiratory chains located in the cytoplasmic membrane. Therefore, the mechanism of cyanide permeability through the cell wall can have a significant role in the respiratory process. In Mitchell's model, from a physical viewpoint, the respiratory process is reduced to the utilization of the energy of the redox reactions [1–4] for the forced transport of protons through the cytoplasmic membrane. When cyanide is added to the electron transport chain of a bacterium, the electron transport, oxygen absorption, and ATP generation are blocked [1], and, thus, the cell stops the breathing. In order to restore the activity, bacteria must somehow eliminate cyanide. This elimination of cyanide is known as the microbial degradation of cyanide [5]. Currently, its physical mechanism has not been elucidated, but experimental work [5] showed that, for the cyanide biodegradation, those biochemical components that are necessary for ensuring the respiratory process in

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Mitchell's model are sufficient. Since there is the confidence that the degradation is caused by the respiratory mechanism, it is obvious that the electron transport is unblocked as a result of the degradation of the cyanide molecule. The real question is how microorganisms can start and carry out the cyanide biodegradation. One of the approaches is to utilize the energy of stored ATP to generate specific enzymes that can degrade cyanide [6, 7]. It is exhausting for microorganisms. However, this mechanism can give an impetus to restore the work of the respiratory chain, which, in turn, will assist in the ATP generation and help to restore other respiratory chains. Since the electron and proton transport chains are associated with the respiratory process, it should be expected that the cyanide biodegradation can be affected by the electromagnetic field. This hypothesis was confirmed in the experimental work [8], according to which, after a short-term irradiation of a cuvette with a suspension of bacteria and cyanide with a weak electromagnetic field with a frequency of 54 MHz, a sharp acceleration of the cyanide biodegradation was observed. The theoretical description at the phenomenological level, which was based on the hypothesis that the acceleration of the degradation is directly related to the activation of the respiratory process by the electromagnetic field, was carried out in work [9], and theoretical and experimental results are in good agreement. Another approach to influencing the cyanide biodegradation is the activation of respiratory centers by an external electric field, as was done in works [10–12]. In particular, it was shown that the short-term treatment with a pulsed electric field stimulates the respiratory activity in the presence of cyanide [13]. An important point is the rate of inhibition of respiratory chains by cyanide in relation to the restoration of their work. Since the restoration of the functionality of the respiratory chain is associated with the degradation of cyanide, the permeability of cyanide molecules through the cell wall at this stage can have a fundamentally important role.

The strains of gram-negative bacteria *Pseudomonas fluorescens* can degrade various types of cyanides [14]. From this viewpoint, the bacterial cyanide degradation has an advantage over the chemical degradation, since specific chemical reactions and processes need to be selected for each type of cyanides or even for a separate cyanide compound. Moreover, after the chemical cyanide degradation, other poisonous compounds may be generated. On the contrary, during the bacterial degradation of cyanides, carbon dioxide and ammonia are created, if bacteria have enough a substrate [5, 15]. For instance, the degradation of free cyanides can be described by the overall reaction [5]:

$$\mathrm{NADH} + 2\mathrm{H}^+ + \mathrm{HCN} + \mathrm{O}_2 \to \mathrm{CO}_2 + \mathrm{NH}_4^+ + \mathrm{NAD}^+.$$

This provides an opportunity for practical applications of the bacterial degradation of cyanide [16]. Note that bacteria capable of decomposing cyanide use it as a source of nitrogen, but cannot use it as a source of carbon [17].

In this article, at the phenomenological level within the framework of the concept of the respiratory mechanism, the impact of the permeability of cyanide molecules through the cell wall on the kinetics of the cyanide biodegradation is investigated considering the short-term treatment with an external pulsed electric field. Based on the comparison of theoretical results with experimental data [13], the analysis of the role of a cell wall in the cyanide biodegradation is carried out.

2. Cyanide Biodegradation and General Phenomenological Model

In the experiment [13], the degradataion of cyanide $\mathrm{Na[Ag(CN)_2]}$ by bacteria *Pseudomonas fluorescens* B5040 was studied. In addition to just a microbial degradation of cyanide, the experiments with the initial short-term pulsed electric field treatment were conducted. The pulsed electric field treatment characteristics are unipolar rectangular impulses with a duration of 1 ms and a frequency of 100 Hz at a voltage of 10, 20, 40, and 70 V. The pulsed electric field treatment duration was 15 min. The duration of measurements of the cyanide biodegradation was 67 h. At the beginning of the experiment, the CN^- concentration in the solution was 19.4 mg/l or 0.74615 mM.

The maximum value of the CN^- concentration at which bacteria *Pseudomonas fluorescens* B5040 can survive in the solution with $\mathrm{Na[Ag(CN)_2]}$ is approximately equal to 100 mg/l [14]. Moreover, the dependence of the oxygen absorption on the CN^- concentration can be considered as a linear dependence at CN^- concentrations below 20 mg/l for the $\mathrm{Na[Ag(CN)_2]}$ compound [14]. For the remainder of this article, we refer to the CN^- concentration of the $\mathrm{Na[Ag(CN)_2]}$ compound as the cyanide concentration.

The presence of cyanide in the periplasmic space of a gram-negative bacteria causes the blockage, or deactivation, of the respiratory chains (RCs) located in the cytoplasmic membrane. For simplicity, we will assume that the cyanide concentration C in the periplasmic space is uniform. Thus, for low cyanide concentrations, the maximum rate of RCs deactivation can be defined as $\gamma(C) = \gamma_0 + \gamma_1 C$, where γ_0 and γ_1 are constants [9]. γ_0 is determined by the natural spontaneous process of stopping of RCs. The actual rate of RCs deactivation is also proportional to the parameter n which is the relative number of active RCs that can degrade cyanide. We consider the parameter n as normalized by the total number of respiratory chains in the system.

During its vital activity, the bacterium itself can restore the functionality of the RCs. Such recovery obviously requires the energy expenditure and depends on the active RCs available in the cell. Thus, the maximum rate of activation of RCs in the simplest case can be defined as $g(n) = g_0 + g_1 n$, where g_0 and g_1 are constants [9]. The actual rate of activation of RCs is also proportional to $(1 - n)$ which is the relative number of blocked RCs.

The impact of the pulsed electric field can be included in the term $D_i(t)(1 - n)$, where the time-dependent parameter $D_i(t)$ characterizes the rate of activation by the external pulsed electric field [9].

Therefore, the overall rate of change in the relative number of active RCs can be represented by the following phenomenological equation [9]:

$$\frac{dn}{dt} = -\gamma(C)\, n + (1 - n)\, g(n) + D_i(t)(1 - n). \quad (1)$$

The maximum rate of cyanide degradation can be determined by the dependence $\alpha(n) = \alpha_0 + \alpha_1 n$, where α_0 and α_1 are constants [9]. The parameter α_0 is related to the cyanide degradation by the chemical compounds in the solution, but such type of degradation was not confirmed in the experiment [13]. Thus, $\alpha_0 = 0$ which means the cyanide can be degraded by active RCs only. For low cyanide concentrations, the actual rate of cyanide degradation is proportional to the cyanide concentration C in the periplasmic space.

The permeation of cyanide into the periplasmic space of a gram-negative bacterium occurs through the pores located in the cell wall of the bacterium [18]. Porins are large enough, and cyanides can diffuse from the extracellular space to the periplasmic space through porins. Thus, the movement of cyanides through the cell wall can be considered as passive diffusion [19].

Therefore, the cyanide concentration changes in the periplasmic space and in the solution can be represented by the following phenomenological equations:

$$\frac{dC}{dt} = -\alpha(n)\, C + \beta_i (C_a - C), \quad (2)$$

$$\frac{dC_a}{dt} = -\beta_a (C_a - C) + g_a. \quad (3)$$

where C is the cyanide concentration in the periplasmic space, C_a is the cyanide concentration in the solution, β_i is the rate of cyanide concentration increase in the periplasmic space because of the diffusion, β_a is the rate of cyanide concentration decrease in the solution because of the diffusion, and g_a is the rate of external cyanide infusion to the solution. In the experiment [13], the parameter g_a is equal to zero, because there is no addition of cyanide to the solution during the measurements. The parameter g_a is introduced in the model to cover additional cases that may be replicated in the experiments.

Thus, the kinetics of cyanide degradation can be described on the basis of the balance equations of the relative number of active respiratory chains and the cyanide concentration present in the solution, accounting for the transition of cyanide from the solution to the periplasmic space through the cell wall. At the same time, active RCs ensure the process of degradation of cyanides and, thus, determine the rate of degradation.

The general phenomenological model (1)–(3) contains several parameters that can be determined only by comparison with the experiment. This may cause some problems, if the number of experimental points is insufficient. For instance, in the experiment [13], there are five main curves that are graphical representations of the experimental data. The first curve is related to only the microbial degradation of cyanide. The other curves are related to the microbial degradation with the initial pulsed electric field treatment at a voltage of 10, 20, 40, and 70 V. If each curve is considered to represent an independent experiment, the number of parameters in the model (1)–(3) will be comparable with the number of experimental data. However, we expect that the number of degrees of freedom will be decreased, because a part of parameters may have the same value for all

curves, or they may have an analytical dependence on the voltage of a pulsed electric field, i.e., each curve is not fully independent. This statement is confirmed for the β_a and β_i parameters based on the approximation obtained below.

3. Stationary Solution

The presence of the term characterizing the infusion of cyanide into the solution in Eq. (3) makes it possible to find a stationary solution of Eqs. (1)–(3) in the case of cyanide degradation by respiratory chains. This immediately follows from Eq. (3) that $C_a = C + g_a/\beta_a$. According to Eq. (2), we obtain $C = \beta_i g_a/\beta_a \alpha_1 n$. In the absence of a pulsed electric field ($D_i = 0$), we obtain the equation for the relative number of active respiratory chains:

$$n^2 + n\left(n_s - \frac{g_0}{g_1}\right) + \left(\frac{\beta_i \gamma_1 g_a}{\beta_a \alpha_1 g_1} - \frac{g_0}{g_1}\right) = 0. \tag{4}$$

The solution of this equation is as follows:

$$n_{1,2} = \frac{1}{2}\left(n_s - \frac{g_0}{g_1}\right) \pm$$
$$\pm \sqrt{\frac{1}{4}\left(n_s - \frac{g_0}{g_1}\right)^2 - \frac{\beta_i \gamma_1 g_a}{\beta_a \alpha_1 g_1} + \frac{g_0}{g_1}}, \tag{5}$$

where $n_s = 1 - \gamma_0/g_1$. The stable stationary solution is $n_{st} = n_1$. The maximum value of the rate of infusion of cyanide into the solution g_a, when the respiratory system is not yet blocked by cyanide and can carry out degradation, is determined under the condition that the values of n_1 must be real, not complex. Thus, we obtain

$$g_{am} = \left[\frac{1}{4}\left(n_s - \frac{g_0}{g_1}\right)^2 + \frac{g_0}{g_1}\right]\frac{\beta_a \alpha_1 g_1}{\beta_i \gamma_1}. \tag{6}$$

Within the model involving pores for the cyanide permeability through the cell wall, we obtain $\beta_a/\beta_i = \Omega_W/\Omega$, where Ω_W is a volume of the periplasmic space of a bacterium, Ω is the average volume of the solution per one bacterium ($1/\Omega$ is the concentration of bacteria in the solution). According to (6), the higher the concentration of bacteria and the larger the volume of the periplasmic space, the greater the performance of the cyanide degradation by bacteria. The ratio $C/C_a = = \beta_i/(\beta_i + \alpha_1 n_1)$ takes small values, if $\beta_i \ll \alpha_1 n_1$, where $\alpha_1 n_1$ is the rate of cyanide degradation by respiratory chains. If $\beta_i \gg \alpha_1 n_1$, cyanide concentrations in the solution and in the periplasmic space are the same.

4. Quasistationary Solution

In the case where there is no infusion of cyanide ($g_a = 0$), which is exactly the case studied in the experiment [13], Eqs. (1)–(3) at $C \neq 0$ do not have a stationary solution. Since the cyanide biodegradation is a slow process. In this case, we can look for a quasistationary solution. We assume that the adjustment of the relative number of RCs is a fast process compared to a change in the concentration of cyanide. Then, from (1) with $g_0 = 0$, $D_i = 0$, and $dn/dt = 0$, we get

$$n = n_s - C(t)\, g_1/\gamma_1. \tag{7}$$

Using (7), Eq. (2), can be transformed into

$$\frac{dC}{dt} = \mu_1 (C_1 - C)(C - C_2), \tag{8}$$

where $\mu_1 = \alpha_1 \gamma_1/g_1$. C_1 and C_2 are solutions of the following equation:

$$-\mu_1 (C_{\rm cr} - C)\, C + \beta_i (C_a - C) = 0, \tag{9}$$

where $C_{\rm cr} = n_s \frac{g_1}{\gamma_1}$. C_1 and C_2 are dependent on time, because C_a is a function of time. Solutions of Eq. (9) are

$$C_{1,2} = \frac{C_{\rm cr} + \xi_1}{2} \mp \sqrt{\frac{(C_{\rm cr} + \xi_1)^2}{4} - \xi_1 C_a}, \tag{10}$$

where $\xi_1 = \frac{\beta_i g_1}{\gamma_1 \alpha_1}$, and $C_{1,2}$ have real values, when $\frac{(C_{\rm cr}+\xi_1)^2}{4\xi_1} > C_a$.

Based on the condition that C_1 and C_2 must be real, we obtain the maximum value of the cyanide concentration in the solution C_a, when the respiratory mechanism is still able to deal with such a high concentration:

$$C_{am} = \frac{(C_{\rm cr} + \xi_1)^2}{4\xi_1}.$$

At the same time, the concentration of cyanide in the periplasmic space will be

$$C_m = \frac{C_{\rm cr} + \xi_1}{2}.$$

From the last correspondence and the condition $C_m \leq C_{\rm cr}$, we obtain $\xi_1 \leq C_{\rm cr}$.

The maximum allowable excess of the concentration from the outside of the bacterium over the concentration in the periplasmic space will be

$$\Delta C = C_{am} - C_m = \frac{C_{\rm cr}^2 - \xi_1^2}{4\xi_1}.$$

Based on the experimental data, $C_{am} \approx 100$ mg/l [14], so, for $C_{\rm cr} \approx 10$ mg/l, we obtain $\xi_1 \approx C_{\rm cr}/40$.

5. Approximate Non-Stationary Solution for the Concentration in the Periplasmic Space

The solution of Eq. (8), which depends on time, in the approximation $C_a = \text{const}$, has the form

$$C(t) = \frac{C_1 + C_2\varphi(t)}{1 + \varphi(t)}, \tag{11}$$

where $\varphi = \exp[-\mu_1 (C_2 - C_1)(t - t_0)]$, and t_0 is defined from the initial conditions. C_1 and C_2 are defined by Eq. (10).

Formula (9) may change in view of the dependence of C_a on time. In the approximation where $\xi_1 \ll C_{\rm cr}$, we will get $C_1 = 0$ and $C_2 = C_{\rm cr}$, and, accordingly,

$$C(t) = \frac{C_{\rm cr}}{1 + \varphi_0(t)}, \tag{12}$$

where $\varphi_0 = \exp[\mu_1 C_{\rm cr}(t - t_0)]$.

6. Solution Approximation for the Cyanide Concentration in the Solution

The formal form of the general solution of Eq. (3) with the initial conditions is

$$C_a(t) = C_a(0)\exp(-\beta_a t) + \\ + \beta_a \exp(-\beta_a t)\int_0^t \exp(\beta_a \tau) C(\tau)\, d\tau. \tag{13}$$

The integral in this equation can be changed to the form

$$\beta_a \int_0^t \exp(\beta_a\tau) C(\tau)\, d\tau = [\exp(\beta_a\tau) C(\tau)]\Big|_0^t - \\ - \int_0^t \exp(\beta_a\tau)\dot{C}(\tau)\, d\tau.$$

To get the solution approximation, the term $\int_0^t \exp(\beta_a\tau)\dot{C}(\tau)\, d\tau$ was excluded. Thus, we obtain the correspondence

$$C_a(t) = [C_a(0) - C(0)]\exp(-\beta_a t) + C(t). \tag{14}$$

Formula (11) can be used as $C(t)$ in Eq. (14), where $C(t)$ depends on $C_a(t)$, which is already a function of time. But, using the condition $\xi_1 \ll C_{\rm cr}$, $C(t)$ takes form (12) which does not contain the explicit dependence on $C_a(t)$.

7. Bacterial Resistance

For the comparison with the experiment in the absence of the action of a pulsed electric field, we will use formula (14), which can be presented in the form

$$C_a(t) = C_w \exp[-\beta_a t] + \frac{C_{\rm cr}}{1 + \exp[\alpha_1 n_s (t - t_0)]}, \tag{15}$$

where $C_w = C_a(0) - C_{\rm cr}/(1 + \exp(-\alpha_1 n_s t_0))$ and the values of $C_{\rm cr}$, $\mu_1 C_{\rm cr} = \alpha_1 n_s$, and t_0 can be found by the comparison with experimental data.

In the simplest case, we use the condition $g_0 = 0$, and the parameter t_0 can be defined by the initial conditions based on correspondence (12).

The actual value of g_0 is not equal to zero, but is very small, $g_0 \ll 1$. Thus, with regard for the bacterial resistance, we can use the condition that follows from (1), when $n = 0$ at the time moment $t = 0$:

$$\left.\frac{dn}{dt}\right|_{t=0} = g_0. \tag{16}$$

Using (7) and (12) and taking a small value of $g_0 \ll \\ \ll 1$, we obtain

$$t_0 = -\frac{1}{\alpha_1 n_s}\ln\left[\frac{g_0}{\alpha_1 n_s^2}\right]. \tag{17}$$

The physical meaning of t_0 is a delay in the degradation of cyanide associated with the action of the resistive mechanism that recovers the activity of the respiratory chains.

8. Electrical Field Impact

To account for the initial short-term treatment of the solution with bacteria by a pulsed electric field at the beginning of the degradation, we use Eq. (1) for which $t \approx 0$ and $n \ll 1$:

$$\frac{dn}{dt} = g_0 + D_i(t). \tag{18}$$

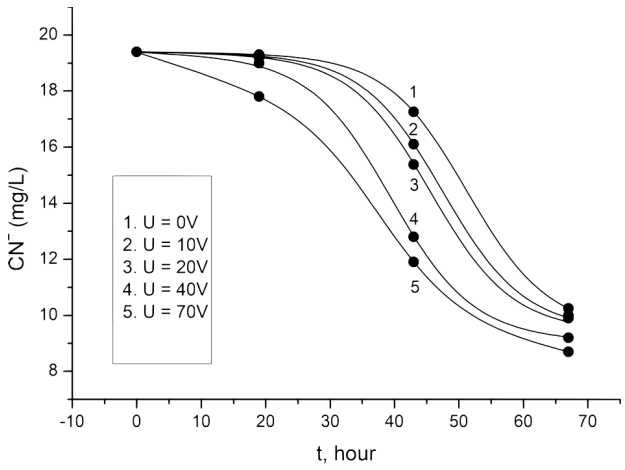

Fig. 1. Dependence of the cyanide concentration on time in the solution with bacteria at different treatment voltages. Solid lines are the result of the calculation. Experimental values [13] are marked with circles

Values of parameters for curves *1–5* in Fig. 1. Calculation errors do not exceed a unit of the last digit in the appropriate parameters

Curve	C_w, mg/L	$C_{\rm cr}$, mg/L	$\alpha_1 n_s$, $\rm h^{-1}$	t_0, h	B	$10^4\beta_a$
1	9.7	9.7	0.16	51.25	0	2.57
2	9.7	9.7	0.16	51.25	0.83	2.90
3	9.7	9.7	0.16	51.25	1.47	3.60
4	9.7	9.7	0.16	51.25	5.63	9.27
5	12.6	6.8	0.16	51.25	7.92	56.30

The impact of a short-term treatment can be included in the parameter t_0 of correspondence (15). Thus, $t_0 = t_0(U)$, if there is a pulsed electric field treatment, and $t_0 = t_0(0)$, if there is no treatment. U is the amplitude of the pulsed electric field. In particular, there is no impact of the treatment at the beginning of the experiment. Thus, $t_0 = t_0(0)$ at the time moment $t = 0$.

We integrate (18) from zero to Δt, where Δt is the duration of the pulsed electric field treatment, and it has a a small value as compared to the time of the observation of a degradation:

$$n(\Delta t) - n(0) = g_0 \Delta t + \overline{D}_i \Delta t, \tag{19}$$

where $\overline{D}_i = \frac{1}{\Delta t}\int_0^{\Delta t} D_i(t)\,dt$.

Using (7) and (17), we obtain

$$\begin{aligned} n(0) &= \frac{\gamma_1}{g_1} C_{\rm cr} \exp\left(-\alpha_1 n_s t_0 (U=0)\right) = \frac{g_0}{\alpha_1 n_s}, \\ n(\Delta t) &= \frac{\gamma_1}{g_1} C_{\rm cr} \exp\left[\alpha_1 n_s \left(\Delta t - t_0(U)\right)\right]. \end{aligned} \tag{20}$$

The correspondence for $n(\Delta t)$ can be reduced to the form

$$n(\Delta t) = \frac{g_0}{\alpha_1 n_s} \exp\left[\alpha_1 n_s \left(\Delta t - t_0(U) + t_0(0)\right)\right]. \tag{21}$$

Using (20) with the condition $\alpha_1 n_s \Delta t \ll 1$, we obtain the equation for $t_0(U)$:

$$t_0 - t_0(V) = \frac{1}{\alpha_1 n_s} \ln\left[1 + \alpha_1 n_s \Delta t + B\right], \tag{22}$$

where $B = \alpha_1 n_s \overline{D}_i \Delta t / g_0$, $\overline{D}_i = d_i U$, d_i is a parameter that may depend on the pulse frequency, $t_0 \equiv t_0(U=0)$. The approximation of B by a linear dependence on the field amplitude is correct for small amplitude values. In view of (22), dependence (15) is modified to

$$C_a(t) = C_w \exp\left[-\beta_a t\right] + \frac{C_{\rm cr}}{1 + (1+B)\exp\left[\alpha_1 n_s (t - t_0)\right]}. \tag{23}$$

At $B = 0$, formulas (15) and (23) coincide.

Thus, the short-term treatment with a pulsed electric field can change the kinetics of cyanide degradation. Such a change will be more noticeable, the larger the amplitude of the pulsed field and the longer the processing time.

9. Comparison with the Experiment

Ratio (23) makes it possible to compare the theory with the experimental values [13] for the degradation of cyanides by bacteria. The comparison was made using the "ORIGIN" program, which allows one to control the quality of the correspondence of theoretical calculations to experimental data.

Figure 1 shows the dependence of the cyanide concentration on time with regard for the permeability of the cell wall and a pulsed electric field treatment at the beginning of the experiment during 15 min at different voltages U. Experimental data are marked with circles, and the theoretical solid lines are obtained as a result of the adjustment of dependence (23) to experimental values. All parameters of Eq. (23) for each curve are listed in Table.

The parameter values for dependences 1–4 turned out to be $C_w = 9.7$ mg/l, $C_{\rm cr} = 9.7$ mg/l, and $\alpha_1 n_s = 0.16$ h^{-1}, $t_0 = 51.25$ h for all curves. However, the best agreement between the experiment and the solution approximation for curve *5* on Fig. 1 is achieved at $C_w = 12.6$ mg/l, $C_{\rm cr} = 6.8$ mg/l. A decrease in the critical value is caused by an increase in the permeability of cyanide to the cell wall according to (10) and $\beta_i \sim \beta_a$.

Figure 2 demonstrates the dependence of the permeability β_a on the voltage amplitude after the treatment of the solution with bacteria by a pulsed electric field. Such a dependence quite clearly demonstrates the exponential form shown in Fig. 2 by a solid line:

$$\beta_a = \beta_{a0} + \beta_{a1} \exp\left(U/U_0\right), \tag{24}$$

where $\beta_{a0} = 1.92 \times 10^{-4}$ h^{-1}, $\beta_{a1} = 5.0 \times 10^{-5}$ h^{-1}, $U_0 = 14.91$ V. The reason for this may be the effect of the field on positively charged nanoparticles. The latter, due to the negative charge of the bacteria, adheres to the surface of the cell wall and closes the pores through which cyanide can diffuse to the cell wall. A pulsed electric field clears the pores from such nanoparticles.

The comparison of experimental and theoretical data makes it possible to determine the dependence of the field influence parameter B on the applied voltage U. This dependence is shown in Fig. 3. The solid line is a linear approximation of $B = d_i U$. As is seen from the figure, a certain non-linearity is observed, but this requires the additional research.

Another point for the additional research is parameter β_a that represents the permeability of cyanide through porins of the cell wall. We found its values based on the comparison of the solution approximation (23) with experimental data [13]. An independent approach to calculate β_a for different bacteria and cyanides needs to be developed. If such calculations demonstrate the matching with the value of β_a in the case where there is no pulsed electric field treatment (curve *1* in Table), it will be an additional evidence that the proposed solution approximation is correct.

On the other hand, during the modelling and comparison of system (1)–(3) with the experiment [13], we identified the critical value $\beta_a^{\rm cr} \approx 2h^{-1}$. During the modeling, if β_a value is greater than $\beta_a^{\rm cr}$, there is no difference between values of the cyanide concentration in the periplasmic space $C\,(t)$ and the cyanide concentration in the solution $C_a\,(t)$ at any point of time; i.e., the cell wall does not have any impact on the cyanide biodegradation. So, the critical value of permeability $\beta_a^{\rm cr}$ is a criterion whether the cell wall has any impact on the cyanide degradation. All β_a values we found during the comparison of the solution approximation (23) with experimental data [13] are less than the critical value of permeability $\beta_a^{\rm cr}$. Thus, the impact of the cell wall needs to be taken into consideration during the analysis of the cyanide biodegradation process.

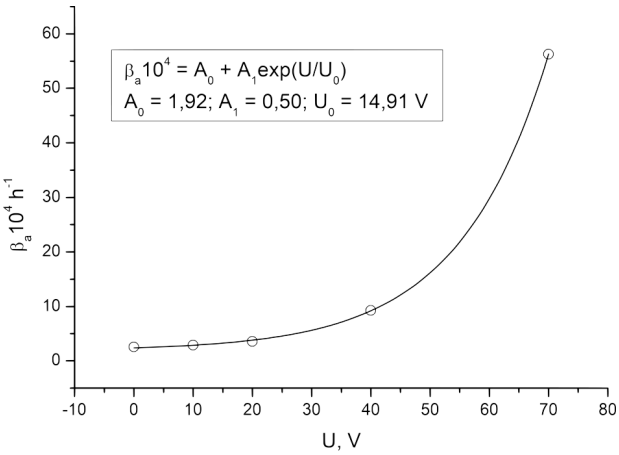

Fig. 2. Dependence of the permeability of cyanide through the cell wall on the amplitude of the pulsed electric field. The solid line is an exponential relationship

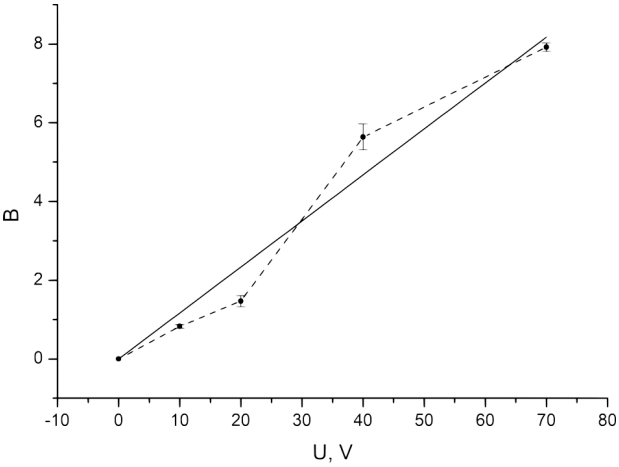

Fig. 3. Dependence of the B parameter on the electric field amplitude. The solid line is a linear approximation

10. Conclusions

Based on the model of respiratory mechanism, the impact of the bacterial cell wall on the cyanide biodegra-

dation process has been confirmed. We have derived the solution approximation for the cyanide biodegradation process and identified the impact of the pulsed electric field treatment on the cyanide permeability through the cell wall. The exponential form of the dependence of the β_a parameter on the voltage of the pulsed electric field is also characteristic of the β_i parameter, because they are proportional. Thus, we decreased the number of the independent parameters in the general model (1)–(3). After determining the value of the permeability β_a for the selected bacteria and a selected cyanide compound, we can identify the appropriate β_a values in the cases where the pulsed electric field treatment is applied using the dependence (24).

We would like to note that an increase in the permeability of cyanide through the cell wall must be obviously accompanied by a higher rate of activation of the respiratory chains. Otherwise, the bacteria would not survive, or, at least, we would not observe an increase in the rate of cyanide biodegradation in the experiments. But, the latter was confirmed in the experiment [13]. Thus, the conducted research partially confirms that the initial short-term treatment by a pulsed electric field shifts the balance in the direction of increasing the rate of activation of the respiratory chains.

Under the conditions of low cyanide concentrations, which were investigated in experiment [13] and in our article, it can be argued that increasing the permeability of cyanide through the cell wall helps the bacteria to neutralize the aggressive environment faster. However, if the concentration of cyanide is high, then an increase in its permeability may lead to the death of bacteria; so, it is not possible to speak unequivocally about the positive effect of the pulsed electric field until the additional experimental and theoretical studies are conducted at high concentrations of cyanides.

We can claim that the steady-state solutions (5) we found, under the condition that cyanide is continuously added to the solution with bacteria, may be used to find the limit of the rate of addition of cyanide (6) that bacteria can still handle with. These results may be used to explain future experiments.

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1. D.L. Nelson, M.M. Cox. *Lehninger Principles of Biochemistry* (WH Freeman, 2012) [ISBN-13: 978-1-4292-3414-6].
2. A.S. Davydov. *Biology and Quantum Mechanics* (Pergamon Press, 1982) [ISBN-13: 978-0080263922].
3. P. Mitchell. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol. Rev. Camb. Philos. Soc.* **41**, 445 (1966).
4. P. Mitchell. Chemiosmotic coupling in energy transduction: A logical development of biochemical knowledge. *J. Bioenerg. Biomembr.* **3**, 5 (1972).
5. R.E. Harris, A.W. Bunch, C.J. Knowles. Microbial cyanide and nitrile metabolism. *Sci. Prog., Oxf.* **71**, 293 (1987).
6. Jui-Lin Chen, D.A. Kunz. Cyanide utilization in Pseudomonas fluorescens NCIMB 11764 involves a putative siderophore. *FEMS Microbiol. Lett.* **156**, 61 (1997).
7. D.A. Kunz, J.L. Chen, G. Pan. Accumulation of alpha-keto acids as essential components in cyanide assimilation by Pseudomonas fluorescens NCIMB 11764. *Appl. Environ Microbiol.* **64**, 4452 (1998).
8. V.I. Podolska, Z.R. Ulberg, V.M. Ermakov *et al.* Study of the effect of low-intensity electromagnetic radiation on biological nanostructures in degradation of transition metal cyanides *Nanosystems, nanomaterials, nanotechnologies* **4**, 245 (2006) (in Ukrainian).
9. V.N. Ermakov, M.M. Kosytskyy. The influence of microwave irradiation to cyanide on bacteria's destruction. *Physics of the Alive* **14**, 11 (2006) (in Ukrainian).
10. V.I. Podolska, V.N. Ermakov, L.N. Yakubenko *et al.* Effect of low-intensity pulsed electric fields on the respiratory activity and electrosurface properties of bacteria. *Food Biophysics* **4**, 281 (2009).
11. Z. Ulberg, V. Podolska, L. Yakubenko, V. Ermakov. Weak pulse electric fields and bacteria respiration. *Proceedings of the International Conference on Bio&Food Electrotechnologies – BFE 2009.* (Imprimerie Danquigny, 2009).
12. V. Podolska, L. Yakubenko, N. Grishchenko *et al.* Influence of weak pulse electric fields on biocolloides-cyanide destructors. *Proceedings of the International Conference on Bio&Food Electrotechnologies – BFE 2009.* (Imprimerie Danquigny, 2009).
13. V.I. Podolska, L.N. Yakubenko, Z.R. Ulberg *et al.* Effect of weak pulse electric fields on surface properties and destructive activity of pseudomonas bacteria. *Colloid Journal* **72**, 830 (2010).
14. L.N. Yakubenko, V.I. Podolska, V.E. Vember, V.I. Karamushka. The influence of transition metal cyanide complexes on the electrosurface properties and energy parameters of bacterial cells. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **104**, 11 (1995).
15. R.E. Harris, C.J. Knowles. Isolation and growth of a Pseudomonas species that utilizes cyanide as a source of nitrogen. *J. Gen. Microbiol.* **129**, 1005 (1983).

16. J.L. Whitlock. Biological detoxification of precious metal processing wastewaters. *Geomicrobiology J.* **8**, 241 (1990).
17. V.M. Luque-Almagro, M.J. Huertas, M. Martinez-Luque *et al.* Bacterial degradation of cyanide and its metal complexes under alkaline conditions. *Appl. Environ. Microbiol.* **71** (2), 940 (2005).
18. R. Benz, K. Bauer. Permeation of hydrophilic molecules through the outer membrane of gram-negative bacteria. Review on bacterial porins. *Eur. J. Biochem.* **176**, 1 (1988).
19. L.M. Shuler, F. Kargi. *Bioprocess Engineering. Basic Concepts* (Prentice Hall PTR, 2002) [ISBN-13: 978-0-13-081908-6].

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ВПЛИВ КЛІТИННОЇ СТІНКИ НА БІОДЕСТРУКЦІЮ ЦІАНІДУ НА ОСНОВІ МОДЕЛІ РЕСПІРАТОРНОГО МЕХАНІЗМУ

На основі моделі респіраторного механізму деструкції ціаніду мікроорганізмами представлено вплив клітинної стінки на процес деструкції в умовах з початковою короткочасною обробкою імпульсним електричним полем та без неї. Дослідження проведено з використанням нелінійних феноменологічних рівнянь і отримано наближений розв'язок. Проведено порівняння теоретичних та експериментальних даних, які добре узгоджуються. Продемонстровано, що початкова короткочасна обробка імпульсним електричним полем збільшує проникливість ціаніду через клітинну стінку, а також швидкість активації дихальних ланцюгів. Стаціонарні розв'язки та максимальна швидкість додавання ціаніду отримані за умов, що ціанід безперервно додається до розчину з бактеріями та немає початкової їх обробки імпульсним електричним полем.

Ключові слова: клітинна стінка, ціанід, імпульсне електричне поле, дихальний ланцюг, порін, проникність клітинної стінки, пасивна дифузія.