Mathematical Modeling of Enzymatic Urea Hydrolysis

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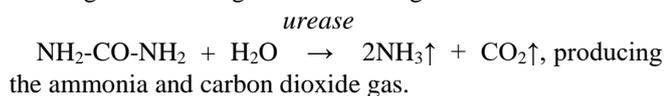
Abstract: The paper deals with the mathematical modeling of biochemical processes underlying the diagnosis of bacteria Helicobacter pylori.
Keywords: urea hydrolysis, Helicobacter pylori (HP), mathematical model, the least square method.

Modeling of enzymatic reactions *in vitro* is one of the effective methods for creating biotechnological production processes, as well as analytical methods involving enzymes. It allows us to distinguish the limiting stage of the process of the biosynthesis of the target products and to evaluate the yield of a key analyte that controls the entire metabolic process.

In this case, the *in vitro* modeling of the biochemical synthesis of ammonia - a key metabolite of *Helicobacter pylori* in its life cycle in the human gastrointestinal tract (GIT) is of great interest for the academic study of the bacteria and practical purposes.

Hydrolysis of urea in the GIT under the influence of urease goes according to the following scheme:

urease

$NH_2\text{-}CO\text{-}NH_2 + H_2O \rightarrow 2NH_3\uparrow + CO_2\uparrow$, producing the ammonia and carbon dioxide gas.

This reaction is analytical and is used to diagnose *HP* in the patient's GIT [1].

We compiled a mathematical model and algorithm of the process, studied the kinetics of urea hydrolysis *in vitro* under by plant urease (*Canavalia ensiformis*) in conditions simulating the deep cultivation of *HP* in the GIT, to optimize the analytical method [2].

The use of plant urease as a model of urease of *HP* is acceptable, since urease is a nickel-containing metalloenzyme (urea amine hydrolase, EC 3.5.1.5) whose active centers are identical irrespective of the source of origin.

Dependence pH of the solution and release of ammonia from time to time were found on the basis of experimental data by approximation using the least square method (OLS).

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Two regression models, in the form of polynomials of second and third degree, were considered for the pH of the solution:

$$y_1(x) = a_0^1 + a_1^1 x + a_2^1 x^2$$
$$y_2(x) = a_0^2 + a_1^2 x + a_2^2 x^2 + a_3^2 x^3$$

We have found the coefficients in dependencies $y_1(x)$ and $y_2(x)$, the sum of the squares of the deviations of calculated data from experimental S_{min}^1 and S_{min}^2, and the hypothesis $a_3^2 = 0$ also was tested by Fisher criteria. The critical point Fischer-Snedecor distribution for significance level $\alpha = 0.1$ is equal to $F_{1,6,0.1} = 3.78$.

Results

$$y_1(x) = 6.839 + 0.043x + 0.021x^2 \quad S_{min}^1 = 0.0024$$
$$y_2(x) = 6.880 + 0.061x + 0.016x^2 + 0.0004x^3 \quad S_{min}^2 = 0.0019$$
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The value of statistics F=1,58. The used result is $y_1(x)$. The experimental data and the calculated curve is presented in figure 1.

Two regression for ammonia models in the form of generalized polynomials of first and second degree were considered.

$$y_1(x) = a_0^1 + a_1^1 e^{-x}$$
$$y_2(x) = a_0^2 + a_1^2 e^{-x} + a_2^2 e^{-2x}$$

The coefficients in dependencies $y_1(x)$ and $y_2(x)$, the sum of the squares of the deviations of calculated data from experimental S_{min}^1 and S_{min}^2 were found and the hypothesis $a_2^2 = 0$ also was tested by Fisher criteria. The critical point Fischer-Snedecor distribution for significance level $\alpha = 0.1$ is equal to $F_{1,7,0.1} = 3.59$.

Results

$$y_1(x) = 243.44 - 204.05e^{-x} \quad S_{min}^1 = 212.33$$
$$y_2(x) = 243.21 - 198.07e^{-x} - 6.23e^{-2x} \quad S_{min}^2 = 210.36$$

The statistics value is F=0,066 .The used result is $y_1(x)$. The experimental data and the calculated curve is presented in figure 2.

CALCULATIONS FOR PH OF THE SOLUTION

x- time, y- solution pH, F(x)- y(x) dependence

574

$$x := \begin{pmatrix} 0 \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7 \\ 8 \\ 9 \end{pmatrix} \qquad y := \begin{pmatrix} 6.88 \\ 6.96 \\ 7.08 \\ 7.23 \\ 7.38 \\ 7.62 \\ 7.92 \\ 8.23 \\ 8.56 \\ 9.00 \end{pmatrix} \qquad n := \text{last}(x) \qquad n = 10 \qquad f(x) := \begin{pmatrix} 1 \\ x \\ x^2 \end{pmatrix}$$

$$i := 1..n$$

$$X^{\langle i \rangle} := f(x_i) \qquad P := X \cdot X^T \qquad V := X \cdot y \qquad a := P^{-1} \cdot V \qquad a = \begin{pmatrix} 6.8931 \\ 0.0428 \\ 0.0211 \end{pmatrix}$$

$$F(x) := a^T \cdot f(x) \qquad S := \sum_i \left(y_i - F(x_i) \right)^2 \qquad S = 0.0024$$

$x_i =$	$y_i =$	$F(x_i) =$	$\left| y_i - F(x_i) \right| =$
0	6.88	6.8931	0.0131
1	6.96	6.957	0.003
2	7.08	7.063	0.017
3	7.23	7.2111	0.0189
4	7.38	7.4013	0.0213
5	7.62	7.6337	0.0137
6	7.92	7.9082	0.0118
7	8.23	8.2248	0.0052
8	8.56	8.5835	0.0235
9	9	8.9844	0.0156

$$M := 2 \qquad k := 1..M \cdot n \qquad h := \frac{10}{M \cdot n} \qquad x0_k := h \cdot (k - 1)$$
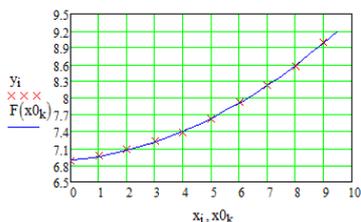

Figure 1. Dynamics of acidity

CALCULATIONS FOR AMMONIA & RESULTS

x- time, y- the ammonia release, F(x)- y(x) dependence

$$x := \begin{pmatrix} 0 \\ 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7 \\ 8 \\ 9 \end{pmatrix} \qquad y := \begin{pmatrix} 38.5 \\ 173.1 \\ 213.4 \\ 225.0 \\ 234.6 \\ 238.5 \\ 244.2 \\ 246.2 \\ 248.1 \\ 250.0 \end{pmatrix} \qquad n := \text{last}(x) \qquad n = 10 \qquad f(x) := \begin{pmatrix} 1 \\ e^{-x} \end{pmatrix}$$

$$i := 1..n$$

$$X^{\langle i \rangle} := f(x_i) \qquad P := X \cdot X^T \qquad V := X \cdot y \qquad a := P^{-1} \cdot V \qquad a = \begin{pmatrix} 243.44 \\ -204.05 \end{pmatrix}$$

$$F(x) := a^T \cdot f(x) \qquad S := \sum_i \left(y_i - F(x_i) \right)^2 \qquad S = 212.33$$

$x_i =$	$y_i =$	$F(x_i) =$	$\left| y_i - F(x_i) \right|$
0	38.5	39.39	0.89
1	173.1	168.37	4.73
2	213.4	215.82	2.42
3	225	233.28	8.28
4	234.6	239.7	5.1
5	238.5	242.06	3.56
6	244.2	242.93	1.27
7	246.2	243.25	2.95
8	248.1	243.37	4.73
9	250	243.41	6.59

$$M := 2 \qquad k := 1..M \cdot n \qquad h := \frac{10}{M \cdot n} \qquad x0_k := h \cdot (k - 1)$$
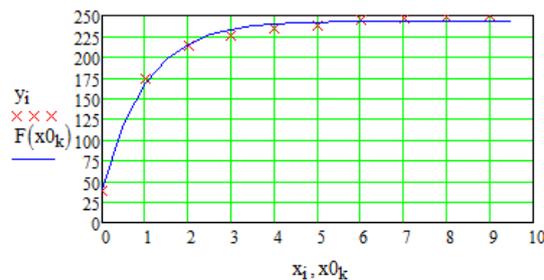

Figure 2. Dynamics of ammonia release

The data obtained are presented in the table and on the graphs 1.2. It follows that the urea hydrolysis with ammonia evolution (main analyte) occurs without diffusion inhibition and the analyte (NH3) to pH≤7.2 goes to neutralize the acidomedium. From pH = 7.2, ammonia is released into the gas phase of the fermenter.

The rate of release of the analyte is proportional to the reaction rate at (t = 0), which indicates an unchanged reaction mechanism until the urea is completely transformed.

A mathematical analysis of the dependence of the pH of the reaction medium on enzymatic hydrolysis of urea on time is considered in two regression models, describing the dynamics of the release of ammonia and changes the pH of culture liquid in the presence of the bacteria *Helicobacter pylori*. The obtained mathematical models are of practical importance as they allow predicting the values of these important biochemical characteristics in randomly selected points in time.

REFERENCES

1. Zhebrun A.B. Infektsiya Helicobacter pylori [Helicobacter pylori infection]. Saint-Petersburg, Fenix Publ., 2006 - 380 p.
2. Dmitrienko M., Parolova N. Invasive and non-invasive biochemical tests for *Helicobacter pylori* diagnosing // Helicobacter. 2016. V. 21, N 1. P. 98.
3. Gmurman V. Theory of Probability and Mathematical Statistics: textbook for applied baccalaureate/12 edition - Publishing house Yrait, 2014 – 479 p. ISBN 978-5-9916-3461-8.