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KINETIC MODEL OF DISSIMILATORY SULFATE REDUCTION

Determination of a kinetic model of sulfates dissimilatory reduction occurring in a lactate nutrient in the presence of bacteria of *Desulfotomaculum ruminis* is presented. It has been found that this process proceeds according to a kinetic model which is based on a system of two consecutive irreversible autocatalytic reactions of the second-order, and its optimum is reached at the temperature of 37°C, pH = 7 and C/S = 9.

1. INTRODUCTION

Process of dissimilatory sulfate reduction involving oxidation of organic substances by microorganisms with simultaneous reduction of sulfates is the source of energy for the organisms depending on anaerobic respiration. The organisms in question are usually bacteria of *Desulfovibrio* and *Desulfotomaculum* species [1], [2]. The reduction process, as has been proved by research carried out in the last couple of years, plays a more and more important role in an efficient biological sewage purification [3]–[7], in waste utilization [8], in recultivation of sulfided soil and various waste-free transformations [9]–[12]. In order to find the optimal conditions for the microorganism activity, it is necessary to investigate the kinetic aspects of this biocatalytic process.

The models which have hitherto been applied proved insufficient to determine basic kinetic parameters characteristic of each of the various ways of this reaction course [13]–[15]. This undoubtedly is a result of the difficulties we encounter when we try to apply the known thermodynamic rules and chemical kinetics to investigation of microorganisms and bacteria cells which are characterized, among others, by changeability and adaptability to any environmental conditions.

This paper presents results of studies on the kinetic model which permits determination of parameters limiting the reaction rate.

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2. MATERIALS AND METHODS

Bacteria from the *Desulfotomaculum ruminis* species were isolated and identified via morphological and physiological methods described earlier [16].

The studies were performed in a tightly sealed reactors containing 50 cm^3 of a nutrient medium. Composition of this medium is as follows: macronutrients (g/dm^3) – $Na_2SO_4 \cdot 10\ H_2O$ – 6.31; $MgSO_4 \cdot 7\ H_2O$ – 2.0; NH_4Cl – 1.0; $CaCl_2$ – 0.13; K_2HPO_4 – 5.0; Mohr salt – 0.006; sodium lactate – 31.4; micronutrients (mg/dm^3) – $Co(NO_3)_2 \cdot 6\ H_2O$ – 5.14; $Ni(NO_3)_2$ – 0.15; Na_2SeO_3 – $1 \cdot 10^{-6}$; $CuSO_4 \cdot 5\ H_2O$ – 12.0; $(NH_4)_2MoO_4$ – 0.99; $Zn(NO_3)_2 \cdot 6\ H_2O$ – 1.0; H_3BO_3 – 8.6; $MnSO_4 \cdot 4\ H_2O$ – 30.8. The nutrient media in the reactors were sterilized at the temperature of 120°C for 20 min, and thereafter helium was passed through the reactor and the bacterial inoculum was itroduced into the nutrient at the ratio of 4%. The temperature of the medium was 37°C, pH = 7, and the C/S ratio amounted to 9. The current concentration of sulfides was measured using the complexometric method which consists in precipitating sulfates with barium chloride, and then in titrating the excess of barium with EDTA [17].

The current concentration of sulfites was determined by means of spectrophotometric measurements of SO_2 absorption at the wavelength of 200 nm. (SO_2 is released from the sulfites after acidification of the medium with sulfuric acid) [18], [19].

Progress in this reaction was controlled by measuring the current concentration of hydrogen sulfide formed during dissimilatory reduction of sulfates. The released hydrogen sulfide was absorbed in washers containing 0.02 M cadmium acetate, and the content of sulfides was determined iodometrically [17].

Bacteria biomass was measured as protein concentration by the Lowry's method [20]. Kinetic calculations, statistical analysis of the data and plots were made using IBM computer with an appropriate software.

3. RESULTS AND DISCUSSION

There are two methods for producing kinetic models, one of them is based on observation of the culture of periodically growing microorganisms, and the second involves determination of the system properties in a stationary state.

Biological systems are very complex, therefore their kinetics can only be approximate in character and can be described on the basis of the models assumed. It is also known that the methods of determining reaction kinetic parameters based on an integrated rate equation are comparable to methods used to determine the initial rates of reactions, taking account of their accuracy as well as repeatability of determinations [21].

As follows from our investigations, the dissimilatory reduction of sulfates (desulfurication) proceeds in two stages, which comprise the reduction of sulfates into sulfites (stage I) and the reduction of sulfites into sulfides (stage II) according to a scheme:

$$SO_4^{2-} \xrightarrow[\text{stage I}]{k_1} SO_3^{2-} \xrightarrow[\text{stage II}]{k_2} S^{2-}.$$

This model is based on experiments performed under previously determined [16] optimal conditions (temperature of 37°C, pH = 7, C/S = 9). In these experiments, except sulfites, no other intermediates were reported to occur [2], as it was postulated by some scientists [2]. The appropriate kinetic curves illustrating the changes in concentration of sulphur compounds in all its forms (SO_4^{2-}, SO_3^{2-}, S^{2-}) occurring during a microbiological reduction are presented in figure 1.

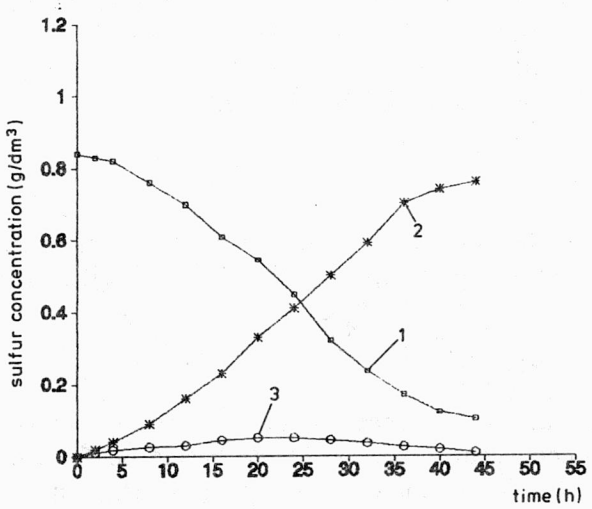

Fig. 1. Kinetic curves of changes in sulfates (*1*), sulfites (*3*) and sulfides (*2*) concentration (temperature of 37°C, pH = 7, C/S = 9)

Given the presented model of microbiological reduction of sulfates, an attempt was made to describe this process by a few, the most probable kinetic equations [16]. If a correlation between a given model and the experimental data was little, such a model was rejected. Thus, six combinations of models, most frequently applied in enzymatic reactions of the zero and first orders, were ruled out. It was found, however, that this process is fairly well described by a kinetic model comprising two consecutive, irreversible autocatalytic reactions of the second-order. The scheme of this model is presented below:

$$\frac{d[A]}{dt} = -k_1[A][X], \tag{1}$$

$$\frac{d[B]}{dt} = k_1[A][X] - k_2[B][X], \tag{2}$$

$$\frac{d[C]}{dt} = k_2[B][X], \tag{3}$$

$[A]$ – sulfate concentration (g S_{SO_4}/dm^3),
$[B]$ – sulfite concentration (g S_{SO_3}/dm^3),
$[C]$ – sulfide concentration (g $S_{S^{2-}}$/dm^3),
$[X]$ – concentration of the bacterial biomass (g/dm^3),
k_1 – rate constant of I stage of sulfate reduction,
k_2 – rate constant of II stage of sulfate reduction,
t – time (hours).

Concentration of bacterial biomass $[X]$, determined in this paper by measuring protein content, in further consideration was replaced by an expression resulting from a solution of the Monod function which illustrates a dependence between a loss of substrate (sulfates) and bacterial biomass increase [22]:

$$\frac{d[A]}{dt} = -\alpha \frac{d[X]}{dt},$$

where α is a coefficient linking sulfate transformation with a bacterial biomass increase.

After integrating this equation, the following expression is obtained:

$$[X] = \frac{[A_0] - [A]}{\alpha} + [X_0],$$

where $[X_0]$ means an initial concentration of bacterial biomass (g/dm^3).

The value of a coefficient α was calculated basing on the curve illustrating the dependence of protein increment on the loss of sulfates with time [16].

After substituting the value of $[X]$ into the kinetic equation (1), we arrive at Bernoulli differential equation, the final solution of which is the following:

$$[A] = \frac{[A_0] + [X_0]\alpha}{1 + \frac{[X_0]\alpha}{[A_0]} e^{k_1\left(\frac{[A_0] + [X_0]\alpha}{\alpha}\right)t}}.$$

A graphic form of the function describing the transformations of sulfates, as well as the correlation of a proper curve with the results of experiments are shown in figure 2a. This figure proves that the correlation coefficient is relatively high, i.e. its value amounts to 0.98.

Using the equation which describes the transformation of sulfates (1) and is derived by nonlinear regression method, constant rates k_1 of stage I of the dissimilatory sulfate reduction as well as the induction period t_1 were determined, provided that $t = t_1$ and $[SO_4^{2-}] = [SO_4^{2-}]_0$. Results of these calculations are compiled in table 1.

Next, the equation of the II stage of the reaction (2), i.e. reduction of sulfites to sulfides, was solved, yielding the following expression:

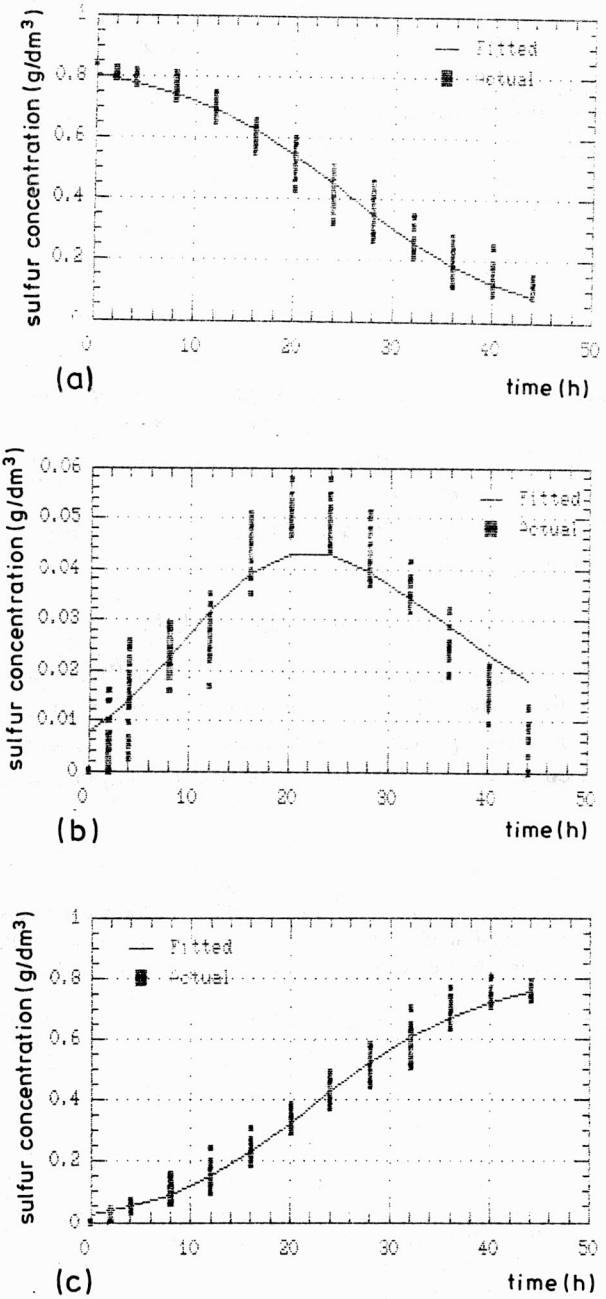

Fig. 2. Correlation between experimental (line) and theoretical data (points) based on the model proposed (temperature of 37°C, pH = 7, C/S = 9)
a – sulfates, b – sulfites, c – sulfides

$$\frac{d[B]}{dt} + k_2[X][B] = -\frac{d[A]}{dt}$$

which is a linear ordinary differential equation of the type [23]:

$$y' + p(t)y = Q$$

where:

$y' = d[B]/dt$,
$y = [B]$,
$p = k_2[X]$,
$Q = -d[A]/dt$.

A graphic representation of the function obtained after solving this equation is shown in figure 2b. Coefficient of the correlation between the curve of stage II of sulfates reduction, i.e. the stage related to transformation of sulfites into sulfides, and experimental data is equal to 0.89.

Solution of the equation permitting determination of sulfides concentration (3) is the following expression:

$$[C] = [A_0] - ([A] + [B]).$$

Inserting the appropriate values of $[A]$ and $[B]$ into this equation, we arrive at a final solution which allows estimating the rate of sulfides formation. The function which is a solution to this equation is shown in figure 2c. Coefficient of correlation between the model curve of sulfides formation and the experimental results is equal

Table

Kinetic parameters and correlation coefficients for the process of dissimilatory reduction of sulfites, calculated on the basis of the kinetic model of two irreversible, autocatalytic subsequent reactions of the II order

$\left(\text{temperature of } 37^\circ\text{C, pH} = 7, \text{ C/S} = 9, \ SO_4^{2-} \xrightarrow[t_1]{k_1} SO_3^{2-} \xrightarrow[t_2]{k_2} S^{2-}\right)$

Sulfates [A]				Sulfites [B]				Sulfides [C]	
k_1 ($dm^3/g \cdot h$)	t_1 (h)	r (SO_4)	F	k_2 ($dm^3/g \cdot h$)	t_2 (h)	r (SO_3)	F	r (S^{2-})	F
0.0532	0	0.98	59671	0.0392	0.6	0.89	3624	0.99	52393

F – coefficient describing relation between the estimation of function variance and the residual variance.

r – coefficient of correlation between experimental data and the model curve describing transformation of sulfates (r_{SO_4}), sulfites (r_{SO_3}) and sulfides (r_S).

k – appropriate constants expressed in ($dm^3/gS \cdot h$).

to 0.99. It should be mentioned here that the subsequent steps describing solutions of successive equations are given both in the doctoral dissertation of DOMAGAŁA [16] and in the annex at the end of this paper.

Coefficients of correlation between the kinetic curves describing reduction of sulfates, sulfites and sulfides and the experimental data as well as F coefficients representing the ratio of function variance to residual variance are listed in the table.

Kinetic parameters calculated for the whole process of sulfate dissimilatory reduction and the respective correlation coefficients are described fairly well by the proposed kinetic model of the desulfurication process. The best agreement is obtained for the intermediate and exponential stages of the process, slightly worse – for the induction stage which is characteristic of microbiological processes. Only in the case of reduction of sulfites to sulfides (stage II of the reduction process), deviation from the model assumed (seen as a declining of the model curve compared with the experimental data) is caused by induction period (t_2). In other cases, however, the calculated coefficients of correlation between model curves and experimental data, as well as always high values of coefficients F suggest that the accepted model consisting of two irreversible, autocatalytic, consecutive reactions of the second-order describes adequately the process of sulfate reduction. It can thus not only be suitable for determining the rates of the whole process and its particular stages, but can also prove a suitable tool for designing the systems fostering an optimal course of the process under industrial conditions.

Given the designed model of desulfurication, in the next paper, we will describe the influence of parameters limiting the rate of this enzymatic process (temperature, pH, concentration of SO_4^{2-}) on its course.

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ANNEX

Solutions of equations are associated with the derived kinetic model of dissimilatory reduction of sulfates (desulfurication). The model accepted involves a system of two consecutive, autocatalytic reactions of the second-order which are described by equations:

$$\frac{d[A]}{dt} = -k_1[A][X], \tag{I}$$

$$\frac{d[B]}{dt} = k_1[A][X] - k_2[B][X] \tag{II}$$

$$\frac{d[C]}{dt} = k_2[B][X] \tag{III}$$

where:

$[A]$ – sulfate concentration (g S_{SO_4}/dm^3),
$[B]$ – sulfite concentration (g S_{SO_3}/dm^3),
$[C]$ – sulfide concentration (g $S_{S^{2-}}$/dm^3),
$[X]$ – concentration of the bacterial biomass (g/dm^3),
k_1 – rate constant of I stage of sulfate reduction,
k_2 – rate constant of II stage of sulfate reduction,
t – time (hours).

Bacterial concentration X was replaced by an expression:

$$[X] = \frac{[A_0]-[A]}{\alpha} + [X_0] \tag{IV}$$

where:

α – coefficient relating a sulfate loss with the protein increment.

This equation is based on the solution of Monod function describing the dependence between the loss of substrate (sulfates) and the increase of bacteria.

1. Solution of equation (I) describing reduction of *sulfates*. By replacing expression (IV) into equation (I) we obtain:

$$\frac{dA}{dt} = -k_1 A\left(\frac{A_0 - A}{\alpha} + X_0\right). \tag{V}$$

After applying the ordering procedure, equation (V) acquires the following form:

$$\frac{dA}{dt} + \underbrace{k_1\left(\frac{A_0}{\alpha} + X_0\right)}_{a} A = \underbrace{\frac{k_1}{\alpha}}_{b} A^2. \tag{VI}$$

The above equation is tantamount to Bernoulli differential equation and thus it is solved after substitution of the following values:

$$z = \frac{1}{A}, \tag{VII}$$

$$z' = -\frac{A'}{A^2}. \tag{VIII}$$

Now, we arrive at a differential linear equation of an ordinary type:

$$z' - az = -b \tag{IX}$$

whose final solution is as follows:

$$A = \frac{a}{b + \text{const} \cdot a \cdot e^{at}}. \tag{X}$$

By inserting exemplary numerical values into the above equation (at pH = 7, temperature of 37°C and C/S = 9) we obtain:

$$A = \frac{0.84624}{1 + 0.0546262 \cdot e^{0.1163997t}}. \tag{XI}$$

2. Solution of equation (II) describing reduction of *sulfites*. After transformation of equation (II):

$$\frac{dB}{dt} = -\frac{dA}{dt} - k_2BX \tag{XII}$$

and introduction of auxiliary variables P and Q, we obtain:

$$\underset{\downarrow P}{\frac{dB}{dt}} + k_2XB = \underset{\downarrow Q}{-\frac{dA}{dt}}. \tag{XIII}$$

This is a differential linear ordinary equation whose solution takes the following form:

$$B = e^{-\int Pdt}\left[\int Q \cdot e^{\int Pdt}dt + \text{const}\right]. \tag{XIV}$$

Solution of the first term of this equation is the expression:

$$e^{\int Pdt} = \left(1 + \frac{X_0\alpha}{A_0} e^{k_1\left(\frac{A_0}{\alpha} + X_0\right)t}\right)^{k_2/k_1} \tag{XV}$$

obtained from equation:

$$\int Pdt = \frac{k_2}{k_1} \ln\left|1 + \frac{X_0\alpha}{A_0} e^{k_1\left(\frac{A_0}{\alpha} + X_0\right)t}\right|. \tag{XVI}$$

Solution of the second term of equation (XIV) is the expression:

$$\int Qe^{\int Pdt} = 0.84624(1 + l \cdot e^{mt})^n \left[\frac{n}{(n-1)(1 + l \cdot e^{mt})} - 1\right] \tag{XVII}$$

where a numerical form of kinetic equation for concentration of sulfates was applied by introducing additional variables:

$l = \text{const}\, X_0 \cdot \alpha / A_0 = 0.055$,

$m = k_1(A_0/\alpha + X_0) = 0.116$,

$n = (k_2 - k_1)/k_1$; $k_1 = 0.053$.

A final solution of equation (II) has the following form:

$$B = \frac{0.85[n - (n-1)(1 + l \cdot e^{mt})]}{(1 + l \cdot e^{mt})^2 (n-1)} + \frac{0.85[(n-1)(1+l) - n](1+l)^{k_2/k_1}}{(1+l)^2(n-1)} \left(\frac{1+l}{1+l \cdot e}\right). \quad \text{(XVIII)}$$

3. Solution of equation (III) describing formation of *sulfides* is obtained by inserting into equation

$$C = A_0 - B - A$$

the expressions for A and B calculated in points 1 and 2.

MODEL KINETYCZNY DYSYMILACYJNEJ REDUKCJI SIARCZANÓW

Przedstawiono wyniki badań nad wyznaczeniem modelu kinetycznego procesu dysymilacyjnej redukcji siarczanów. Proces ten zachodzi z udziałem bakterii *Desulfotomaculum ruminis* w pożywce mleczanowej. Ustalono, że proces ten odbywa się według modelu kinetycznego, którego podstawą jest układ dwóch następczych, nieodwracalnych reakcji autokatalitycznych drugiego rzędu; jego optimum przypada na temperaturę 37°C, pH = 7 i C/S = 9.

КИНЕТИЧЕСКАЯ МОДЕЛЬ ДИССИМИЛЯЦИОННОЙ РЕДУКЦИИ СУЛЬФАТОВ

Представлены результаты исследований определения кинетической модели процесса диссимиляционной редукции сульфатов. Этот процесс протекает с участием бактерий *Desulfotomaculum ruminis* в лактатной питательной среде. Было установлено, что этот процесс протекает согласно кинетической модели, которой основой является система двух последовательных, необратимых автокаталитических реакций второго порядка; оптимальные условия этого процесса: температура 37°C, pH = 7, C/S = 9.