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KINETIC MODEL OF DENITRIFICATION BY *BACILLUS* BACTERIA

We present the kinetic studies of denitrification brought about by *Bacillus* genus in the medium containing lactate as the only organic substrate. The results suggest that denitrification can be expressed as a sequence of two consecutive second-order reactions.

1. INTRODUCTION

Kinetic models of denitrification proposed so far [1]–[3] are insufficient to calculate kinetic parameters for different conditions [4]–[6]. Difficulties mainly arise when the concentrations of bacterial cells and organic products of the reaction are concerned. Among the parameters affecting denitrification, organic substrate is of great interest and is specially important in wastewater neutralization. In some cases, there is no need to introduce additional organic carbon compound when the waste itself contains easily degradable carbon. In this paper, the kinetics of denitrification brought about by *Bacillus* genus was investigated. The media containing lactate as the only organic substrate were applied.

The entire process of denitrification can be expressed by the following scheme:

$$NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2.$$

The reduction of NO to N_2O and further to N_2 is extremely fast in relation to the first two stages, so we based our model on a simplified scheme:

$$NO_3 \rightarrow NO_2 \rightarrow N_2$$

which can be treated as a sequence of two consecutive reactions with stable intermediate.

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2. MATERIALS AND METHODS

Bacterial cultures, identified as *Bacillus* genus, were grown in the glass, air-tight reactors at 37°C. Growth medium (all concentrations are expressed in g/dm^3): KNO_3 – 10 ($N_{NO_3^-}$ = 1.4), KNO_2 (medium does not contain nitrate) – 1.07; 2.01 ($N_{NO_2^-}$ = 0.175; 0.33), $Fe(NO_3)_3 \cdot 9H_2O$ – 0.44, $MgSO_4 \cdot 7H_2O$ – 0.5, $Na_2HPO_4 \cdot 12H_2O$ – 2.5, NH_4Cl – 0.25, $CaCl_2$ – 1, sodium lactate – 7.84 (C = 2.52) and microelements [3].

Nitrate concentration was measured potentiometrically using ion-selective electrode "Detector". Nitrite concentration was measured colorimetrically [7] on Specord VSU2P, Carl Zeiss, Jena. Protein concentration was measured according to Lowry method [8].

3. RESULTS AND DISCUSSION

Initially, for denitrification we tried to introduce the model of first order reactions with induction time. It turned out, however, that correlation between the results obtained and the experimental data was low. Higher correlation was obtained for the model of zero order reactions. The results of this approach are shown in figure 1 and in table 1.

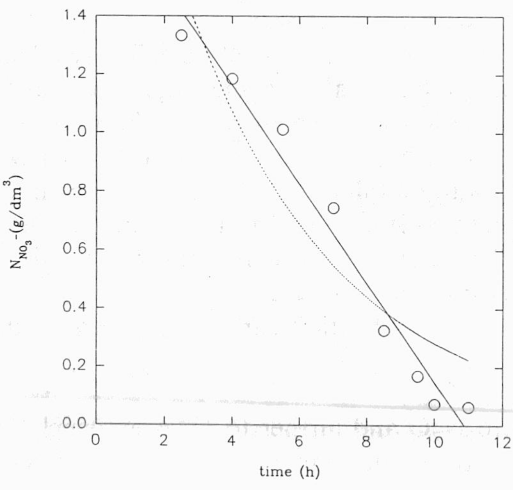

Fig. 1. Experimental data and theoretical curves representing nitrate reduction (C/N = 1.8, pH = 7, temp. = 37°C)
o – experimental data, —— – zero order kinetic curve, --- – first order kinetic curve

The sigmoid shape of the curve representing nitrate reduction suggests that the best overlapping of curves might be obtained based on the model of the reaction, described by the following equations:

Table 1

Rate constants (k), induction times (t) and correlation coefficients (r) for first and zero order kinetic models of nitrate reduction ($C/N = 1.8$, $pH = 7$, temp. = 37°C)

First order reaction			Zero order reaction		
k (h^{-1})	t (h)	r	k (g $N_{NO_3^-}/dm^3h$)	t (h)	r
0.23 ± 0.002	2.8 ± 0.2	0.89	0.170 ± 0.007	2.5 ± 0.2	0.96

$$[NO_3^-] + aX \underset{t_1}{\overset{k_1}{\rightarrow}} [NO_2^-] + 2aX, \tag{1}$$

$$[NO_2^-] + bX \underset{t_2}{\overset{k_2}{\rightarrow}} \frac{1}{2}[N_2] + 2bX, \tag{2}$$

k_1, k_2 – rate constants of nitrate and nitrite reductions, respectively,
t_1, t_2 – induction times of nitrate and nitrite reductions, respectively,
X – protein concentration (g/dm^3),
a, b – coefficients combining nitrate and nitrite reductions, respectively, with protein growth.

The rate of nitrate and nitrite reduction and the increase of protein concentration can be expressed as follows:

$$-\frac{dA}{dt} = k_1 AX, \tag{3}$$

$$\frac{dB}{dt} = k_1 AX - k_2 BX, \tag{4}$$

$$\frac{dX}{dt} = ak_1 AX + bk_2 BX, \tag{5}$$

A – nitrate concentration (g $N_{NO_3^-}/dm^3$),
B – nitrite concentration (g $N_{NO_2^-}/dm^3$).

Based on equations (1), (2), the protein concentration can be described as follows:

$$X = X_0 + aB + (a+b)(0.5[N_2]), \tag{6}$$

$$0.5[N_2] = A_0 - A - B, \tag{7}$$

therefore

$$X = X_0 + (a+b)A_0 - (a+b)A - bB. \tag{8}$$

The nitrite concentration can be expressed as function of nitrate concentration:

$$\frac{dB}{dA} = -1 + \frac{k_2}{k_1 A} B. \tag{9}$$

Equation (9) after integration can be expressed as follows:

$$B = \frac{1}{c-1}(A - A_0^{1-c}(A)^c), \quad c = k_2/k_1. \tag{10}$$

Coefficients a and b were calculated from the equation describing protein concentration as the function of nitrate changes, which was obtained as a result of combining equations (8) and (10)

$$X = pA^c - nA + l, \tag{11}$$

$$p = \frac{b}{c-1} A_0^{1-c},$$

$$n = \frac{c(a+b) - a}{c-1},$$

$$l = X_0 + (a+b)A_0.$$

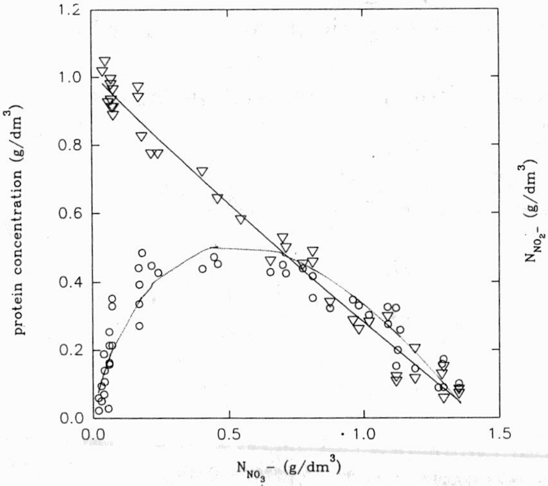

Fig. 2. Relation between protein and nitrate concentrations and nitrite and nitrate concentrations (C/N = 1.8, pH = 7, temp. = 37°C)
▽ – protein concentration, experimental data, — – curve expressed by equation (11),
○ – nitrite concentration, experimental data, ····· – curve expressed by equation (10)

Relations between protein and nitrate concentrations and nitrite and nitrate concentrations are shown in figure 2.

The kinetics of the process in the growth media containing nitrite, but not nitrate, has been studied. The model proposed was described by the following equations:

$$[NO_2^-] + bX \underset{t_2}{\overset{k_2}{\rightarrow}} 0.5[N_2], \tag{12}$$

$$-\frac{dB}{dt} = k_2 BX, \tag{13}$$

$$X = X_0 + b(B_0 - B). \tag{14}$$

Kinetic parameters were calculated based both on expression (13) and zero order reaction. The results are given in table 2 and in figure 3. In both cases we got satisfactory correlation between theoretical and experimental data. The value of coefficient b changes with the initial concentration of nitrite, indicating that nitrite can be an electron acceptor in the system studied, although it is not as efficient as nitrate.

Table 2

Rate constants (k), induction times (t) and correlation coefficients (r) for nitrite reduction in nitrite medium ($C_{org} = 2.52$ g/dm^3, pH = 7, temp. = 37°C)

$[N_{NO_2^-}]_0$	Second order reaction				Zero order reaction		
(g/dm^3)	k (dm^3/g of protein/h)	t (h)	b	r	k (g $N_{NO_2^-}$/dm^{3}h)	t (h)	r
0.175	11.66±0.77	2.27±0.13	0.32	0.98	0.030±0.001	1.95±0.14	0.99
0.33	3.33±0.18	1.60±0.18	0.41	0.98	0.0262±0.0006	1.46±0.17	0.99

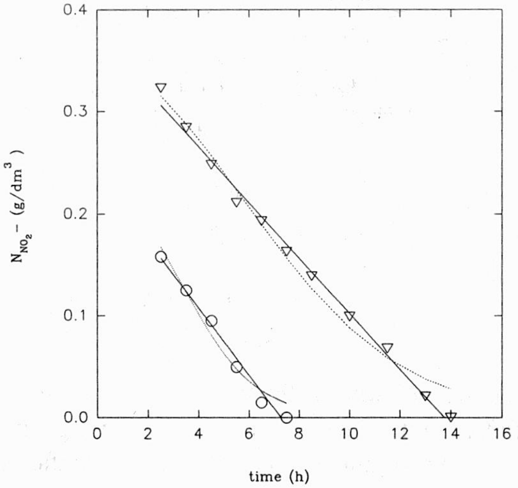

Fig. 3. Experimental data and theoretical curves representing reduction of nitrite in nitrite medium (pH = 7, temp. = 37°C)
o – experimental data for $[N_{NO_2^-}]_0 = 0.175$ g/dm^3, ▽ – experimental data for $[N_{NO_2^-}]_0 = 0.33$ g/dm^3, — – kinetic curve for zero order reaction, ···· – kinetic curve for autocatalytic model

Finally, from the equations (3), (4), (5) and (11) we got the following expressions:

$$-\frac{dA}{dt} = k_1 A(pA^c - nA + l), \tag{15}$$

$$\frac{dB}{dt} = k_1 A(pA^c - nA + l) + k_2 B(pA^c - nA + l), \tag{16}$$

$$\frac{dX}{dt} = ak_1 AX + bk_2 BX. \tag{17}$$

The above equations were integrated. As expressions (15) and (17) have not got analytical solution, they were approximated. The ratio $c = k_1/k_2$ was equal to 1.03, therefore function A^c was replaced by the function $gA + r$. In this case, the set of equations can be expressed as follows:

$$A = \frac{wA_0 \exp(-wk_1(t-t_1))}{z - sA_0 \exp(-wk_1(t-t_1))}, \tag{18}$$

$$B = \frac{1}{c-1}\left\{\frac{wA_0 \exp(-wk_1(t-t_1))}{z - sA_0 \exp(-wk_1(t-t_1))} - \left[A_0^{1-c}\left(\frac{wA_0 \exp(-wk_1(t-t_1))}{z - sA_0 \exp(-wk_1(t-t_1))}\right)^c\right]\right\},$$

$$X = X_0\left\{\exp\left(-\left(\frac{uk_1k_2(t-t_1)}{k_2-k_1}\right)\right)\left[\frac{z - sA_0 \exp(-(wk_1(t-t_1))}{w}\right]^{(m)}\right\},$$

$$w = \frac{brA_0^{1-c}}{c-1} + X + (a+b)A_0,$$

$$s = \frac{bgA_0^{1-c} - c(a+b) + a}{c-1},$$

$$z = sA_0 + w,$$

$$u = brA_0^{1-c},$$

$$m = \frac{a(k_2-k_1) - (1-gA_0^{1-c})bk_2}{s(k_2-k_1)}.$$

The kinetic parameters, calculated on the basis of the above approach, their standard deviations and correlation with the experimental data are given in table 3 and figure 4.

The results of our investigations suggest that denitrification brought about by *Bacillus* in lactate medium can be expressed as a sequence of two consecutive second order reactions. The proposed kinetic model of denitrification can be of great importance to both further basic studies of the process and to biotechnology in environmental protection.

Table 3

Kinetic parameters of denitrification based on the kinetic model proposed (C/N = 1.8, pH = 7, temp. = 37°C)

Nitrate			Nitrite		Protein			
k_1	t	r	k_2	r	k_1	k_2	t	r
0.66 ± 0.04	1.8 ± 0.3	0.98	0.67 ± 0.02	0.91	0.71 ± 0.02	0.72 ± 0.02	1.4 ± 0.2	0.98

k_1, k_2 – rate constants ($dm^3/g\,h$), t – induction time (h), r – correlation coefficients.

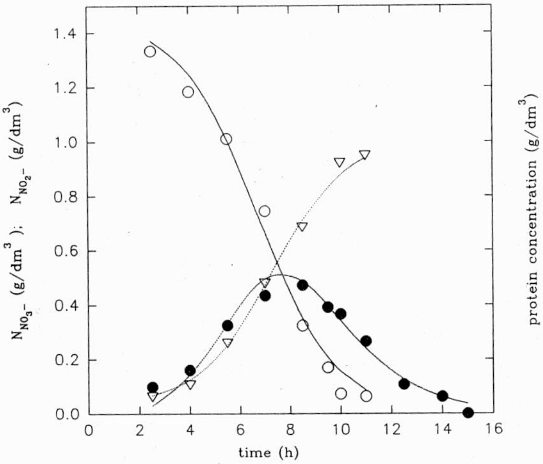

Fig. 4. Correlation between experimental and theoretical data for the kinetic model proposed (C/N = 1.8, pH = 7, temp. = 37°C)
o – nitrate concentration, experimental data, ● – nitrite concentration, experimental data, ▽ – protein concentration, experimental data, — – theoretical curve of nitrate reduction, ···· – theoretical curve of protein increase, --- – theoretical curve of nitrite concentration

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MODEL KINETYCZNY DENITRYFIKACJI ZACHODZĄCEJ Z UDZIAŁEM BAKTERII *BACILLUS*

Przedstawiono badania kinetyki reakcji denitryfikacji prowadzonej przez bakterie *Bacillus* w pożywce mleczanowej. Uzyskane wyniki wskazują, że denitryfikacja może być dobrze opisana przez model dwóch reakcji następczych drugiego rzędu.

КИНЕТИЧЕСКАЯ МОДЕЛЬ ДЕНИТРИФИКАЦИИ, ПРОИСХОДЯЩЕЙ С УЧАСТИЕМ БАКТЕРИЙ *BACILLUS*

Представлены исследования кинетики рекции денитрификации, происходящей с участием бактерий *Bacillus* в лактатной среде. Полученные результаты показывают, что денитрификацию можно хорошо описать посредством модели двух последующих реакций второго порядка.