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TEMPERATURE DEPENDENCY OF BIOLOGICAL DENITRIFICATION WITH ORGANIC MATERIALS ADDITION

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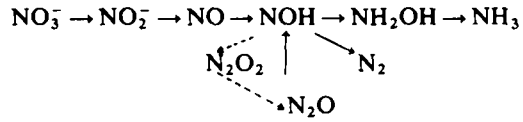
Abstract—The rate of a waste water dentification process depends amongst others upon the temperature and the kind of compound used as electron donor. The results of measurements of denitrification kinetics using methanol, acetone and acetic acid have been presented.

INTRODUCTION

In recent years much attention has been given to biological denitrification. Many investigators have examined factors influencing denitrification sequence, i.e. anoxic conversion of nitrate through nitrite to elemental nitrogen gas. Dissimilatory nitrate metabolism occurs in a series of enzyme catalyzed reactions similar to the route hypothesized by Fewson & Nicholas (1961):



—— enzymatic
--- non-enzymatic.

The reaction sequence is partially confirmed through the enzymes found in denitrifying bacteria: nitrate reductase, nitrite reductase, nitric oxide reductase, hyponitrite reductase (not very well established), and hydroxylamine reductase (Christensen & Harremoes, 1972). In these reactions nitrate is reduced by serving as a hydrogen acceptor. An additional, but a relatively small quantity of nitrate is reduced to ammonia in order to supply nitrogen for cell synthesis. Typical denitrification genera listed by Painter (1970) are *Pseudomonas* sp., *Micrococcus* sp., *Spirillum* sp. and *Achromobacter* sp. In the absence of molecular oxygen these organisms use nitrate or nitrite as terminal electron acceptors while oxidizing organic matter for energy.

For application in wastewater treatment the denitrification process must operate over a wide range of temperatures. The variation in the denitrification rate, expressed in terms of the various temperature employed is given, according to Sutton *et al.* (1975), in Table 1. The denitification rate depends not only on the temperature but amongst others the kind of compound utilized as electron donor. The effect of denitrification by means of activated sludge at different temperatures using methanol, acetone and acetic acid as electron donors were examined.

KINETIC CONSTANTS

In non-limiting nutrient systems the dentirification reaction is the zero order. The reaction rate is given by:

$$k = \frac{dc}{dt} \tag{1}$$

where:

k = reaction rate, mg l^{-1} h^{-1}
c = substrate concentration, mg l^{-1}
t = time, h.

The effect of temperature on the reaction rate can be expressed by a temperature coefficient Q_{10}:

$$Q_{10} = \frac{k_{t+10}}{k_t} \tag{2}$$

where:

k_t = reaction rate at temperature t, mg l^{-1} h^{-1}
k_{t+10} – reaction rate at temperature $t + 10$, mg l^{-1} h^{-1}.

The value of Q_{10} is only approximate because it depends on the range of temperatures over which the measurement was done. Reaction rate is usually evaluated at 20°C. The relationship between the measured values and the reaction rate at 20°C is:

$$k_{20} = \frac{k_t}{\theta^{t-20}} \tag{3}$$

where:

θ = constant.

The temperature effect on the reaction rate is indicated by the value of the activation energy in the Arrhenius equation:

$$k = A \cdot e^{-E_a/RT} \tag{4}$$

Table 1. Temperature coefficients and denitrification (Sutton *et al.*, 1975)

	Temperature range (°C)	Arrhenius activation energy (E_a = cal mol^{-1})	θ Values	Q_{10} values ($Q_{10} = \theta^{10}$)	Reference
Activated sludge	6–25	15,900	1.09		Sutton (1973)
SRT = 6 days	6–16			2.5	Pilot plant
	10–20			2.5	
Upflow packed	5–25	11,090	1.07		
column	5–15			2.1	
	10–20			2.0	
Batch	3–27	16,800	1.12		Dawson & Murphy
P. denitrificans	10–20		1.10	3.0	(1972) Lab scale
Batch	15–25	10,000	1.06	1.74	Stensel (1970)
Activated sludge					Lab scale
SRT = 2 days					
Continuous					
activated sludge	10–20	19,500	1.13	3.3	
Activated sludge	10–20	19,000	1.15	3.3	Mulbarger *et al.*
SRT = 7.6 days					(1970) Pilot plant
Activated sludge	10–20			2	Johnson & Vania
					(1971) Pilot plant
Batch	10–20			2.6	Wuhrman &
Activated sludge					Mechsner (1965)
					Lab scale

where:

A = frequency factor
E_a = activation energy, cal mol^{-1}
R = universal gas constant, cal mol^{-1} deg^{-1}
T = absolute temperature, K.

With the use of a logarithmic transformation we could obtain a linearized form of the Arrhenius equation:

$$\ln k = \ln A - \frac{E_a}{RT}. \tag{5}$$

This model, using decimal logarithmic scale, was fitted to the experimental data using the least squares fit. Calculation of the slope for the linearized model provided an evaluation of the Arrhenius activation energy:

$$E_a = \frac{4.576 \cdot T_1 \cdot T_2}{T_1 - T_2} \log \frac{k_1}{k_2}. \tag{6}$$

As the reaction rate in biological reactors depends on the microorganisms concentration it was taken into consideration that the relative specific reaction rate is:

$$k' = \frac{k}{X} \tag{7}$$

where:

k' = relative specific reaction rate, h^{-1},
k = measured reaction rate, mg l^{-1} h^{-1}
X = concentration of microorganisms, mg l^{-1}.

EXPERIMENTAL PROCEDURE

Fresh activated sludge was obtained from the Vienna–Blumental Sewage Treatment Plant which works according to the simultaneous nitrification–denitrification process (Matsche, 1977). In the treatment plant wastewater of about 200,000 PE is treated without primary sedimentation in aeration tanks with mammoth rotor aeration.

Generally in each experiment the activated sludge was transferred into 1 l. plastic containers which were placed in a thermostatic bath. Complete mixing was accomplished by means of magnetic stirrer. The experiments were carried out at 2.5, 5, 10, 15, 20, 25, 30 and 35°C. Before the measurement the samples were moderately aerated and adapted to the temperature of measurement for 2 h. After 2 h a solution of 10 g l^{-1} KNO_3 was added to each of the samples to obtain a concentration of about 30 mg l^{-1} N–NO_3 within the liquor. The nitrate concentration was measured by means of ion-selective electrode. Next, the solution of methanol, acetone and acetic acid were added respectively. The stoichiometric reactions during denitrification are as follows:

with addition of methanol:

$$NO_3^- + \tfrac{5}{6}\,CH_3OH = \tfrac{1}{2}\,N_2 + \tfrac{5}{6}\,CO_2 + \tfrac{7}{6}\,H_2O + OH^-$$

with addition of acetone:

$$NO_3^- + \tfrac{5}{16}\,CH_3COCH_3 = \tfrac{1}{2}\,N_2 + \tfrac{15}{16}\,CO_2 + \tfrac{7}{16}\,H_2O + OH^-$$

with addition of acetic acid:

$$NO_3^- + \tfrac{5}{8}\,CH_3COOH = \tfrac{1}{2}\,N_2 + \tfrac{5}{4}\,CO_2 + \tfrac{3}{4}\,H_2O + OH^-.$$

The concentration of the added reagents were respectively: methanol 75 mg l^{-1}, acetone 65 mg l^{-1}, acetic acid 85 mg l^{-1} within the liquor. Aeration was stopped and the oxygen consumption was observed by means of oxygen electrode. The measurement began 5 min after obtaining the anoxic conditions and the samples were collected every 30 min for 3 h in the case of low temperatures and every 10 min for 1 h at higher temperatures. Samples were filtered through filter paper (Schleicher und Schüll No. 595) and the concentration of N–NO_3 was measured by use of a Technicon analyzer. The concentration of microorganisms was measured as MLSS.

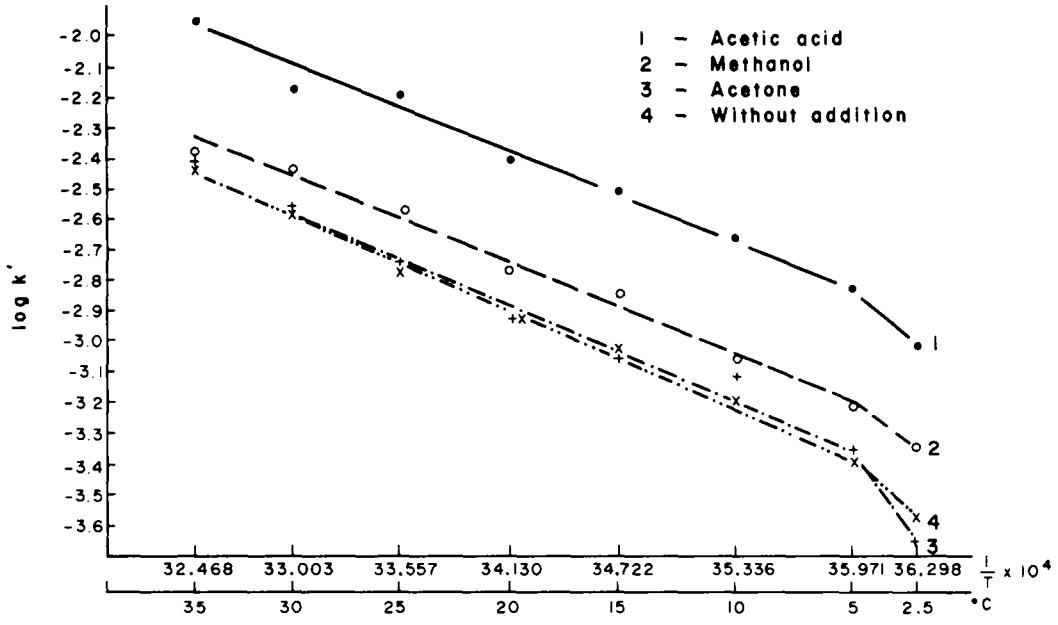


Fig. 1. Arrhenius dependence of rate constant for denitrification with addition of synthetic carbon source.

RESULTS

Figure 1 shows the results of a study. From these data the kinetic constants were found and presented in Table 2.

The data shows that the relationship between specific reaction rate and temperature within the range 5–35°C is linear. Below 5°C, the slowdown in reaction rate is greater that could be expected on the basis of results obtained in the range 5–35°C. The least squares fit for the data in this range is as follows:
With addition of methanol:

$$\log k' = -2422\frac{1}{T} + 5.5288 \qquad r = 0.9927$$

With addition of acetone:

$$\log k' = -2602\frac{1}{T} + 6.0109 \qquad r = 0.9871$$

With addition of acetic acid:

$$\log k' = -2369\frac{1}{T} + 5.7083 \qquad r = 0.9906$$

Without any addition:

$$\log k' = -2677\frac{1}{T} + 6.2472 \qquad r = 0.9948.$$

The sample without any organic chemical addition shows relatively high denitrification rate. This is probably caused by the type of plant used at the Vienna–Blumental Treatment Plant. The Plant works without primary sedimentation. The wastewater after removal of coarse material by bar screens and withdrawal of sand and flotable material in an aerated grit chamber, flows directly to the aeration tanks which no doubt results in more than the normal amount of organic matter being adsorbed onto the flocs and so causing the high rate of exogenous denitrification.

Acetic acid was most effective at increasing the denitrification rate followed by methanol, whilst the use of acetone showed no improvement over the undosed sample. The scale of improvement can be shown by simple comparison. The denitrification rate with addition of acetic acid at 5°C is approximately the same as that at 23°C without any addition.

Table 2. Kinetic constants

Chemical	$k'_{20}\cdot 10^3$ (h^{-1})	$k'_{10}\cdot 10^3$ (h^{-1})	Q_{10}	$\theta = \sqrt[10]{Q_{10}}$	E_a (cal mol^{-1})
Without addition	1.29	0.61	2.11	1.08	12,300
Methanol	1.83	0.93	1.97	1.07	11,200
Acetone	1.35	0.66	2.05	1.07	11,800
Acetic acid	4.20	2.17	1.94	1.07	10,900

It is well known that the relationship between the specific reaction rate and the temperature is, in case of the enzyme catalyzed reactions, linear only in a relatively narrow range. The results which were obtained show that the lower point of inflexion is below 5°C and does not depend on the kind of organic compound used for improving the amount of denitification achieved.

The Arrhenius activation energy E_a was in all cases more or less the same and varied in the range 10.9–12.3 kcal mol^{-1}. Parameter Q_{10} changes in the range 1.94–2.11 and does not depend on the type of organic materials consumed. This shows evidently that in all cases the reaction course according to the van't Hoff rule. The comparison of obtained results with the data presented in Table 1 shows that the kinetic constants like Q_{10}, θ and E_a only vary slightly, and lie about the theoretical values obtained from the van't Hoff rule.

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