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Original Research

Microbiocide Efficiency of APDA in Denitrification and Desulfurication Processes

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Abstract

The effect of concentration of N,N-Bis(3-amine propyl)dodecylamine (APDA) used in disinfectants (Lonzabac) on the kinetics of denitrification and desulfurication processes proceeding with the use of *Bacillus licheniformis* and *Desulfotomaculum ruminis* bacteria, was studied.

The kinetic equation for denitrification in a medium containing the microbiocide tested was derived, the parameters of the process were calculated, and the toxicity limits of APDA were established. The latter are of importance when considering means for environmental protection against APDA effects on nitrogen and sulphur cycles.

Keywords: *Bacillus licheniformis* bacteria, *Desulfotomaculum ruminis,* toxicity, denitrification, desulfurication, APDA, reaction order, kinetic model of denitrification

Introduction

Microbiocidic amine APDA has recently been frequently applied as an attractive and effective disinfectant known on the market as among other names, Lonzabac [1]. It is active against bacteria, fungi, and viruses, so preparations containing this compound have been used in hospitals, the food industry, the cosmetic industry, and in household products [1]. The range and antimicrobiological activity of APDA depend on the concentration, time of contact, temperature and pH of the environment, and of course on the kind of microorganisms in a given ecosystem. Of particular importance are the problems related to neutralization and management of wastes left after the use of preparations containing APDA. The common use of APDA as a disinfectant prompted us to study the effect of this substance on the biological cycles of nitrogen and sulphur in the ecosystem. The aim of this study was to determine the degree of biodegradation and toxic concentrations of APDA to prevent any possible harmful effects on the environment. The results are of interest to ecologists and eco-toxicologists.

This paper reports the influence of APDA on the kinetics of microbiological reduction of nitrates [2] and sulphides [3] proceeding with the involvement of *Bacillus* and *Desulfotomaculum* bacteria, and determines of maximum admissible and toxic concentrations of APDA.

Materials and Methods

Denitrification

Bacteria from the genus *Bacillus* were isolated and identified in the way described earlier [4].

Kinetic Studies

Were conducted at 37°C at pH 8 in tightly closed glass reactors of 20 cm^3 capacity, containing 10 cm^3 of the lactate medium composed of [g/dm^3]: $N\text{-}NO_3^-$ = 1.40, $Fe(NO_3)_3 \times 9H_2O$ = 0.44, NH_4CI = 0.25, $MgSO_4 \times 7H_2O$ = 0.50, $CaCl_2$ = 1.00, $Na_2HPO_4 \times 12H_2O$ = 2.50, C_{org} = 3.27 and microelements [5]. The medium was inoculated with 4% of the inoculum collected after 24 h of bacteria growth (the phase of logarythmic growth). Then, an appropriate amount (to a maximum concentration of 10 ppm) of the tested APDA was added to the reactors. The rate of denitrification was determined by measuring the concentration of nitrates, nitrites and proteins (from the bacteria biomass) at certain time intervals.

Desulfurication

The bacteria-reducing sulphates were isolated and identified as *Desulfotomaculum ruminis* by the method described earlier [6].

Kinetic Studies

Were carried out at 37°C in anaerobic conditions (helium) at pH 6.8-7.2, in sealed glass reactors containing 50 cm^3 of sterilized modified Starkey medium composed of [g/dm^3]: $MgSO_4$ x $7H_2O$ = 2.00, Na_2SO_4 = 2.66, NH_4CI = 1.00, K_2HPO_4 = 5.00, $CaCl_2$ = 0.13, Mohr salt = 0.006, sodium lactate = 25.00, and microelements [5]. Having added an appropriate amount of the tested APDA in concentrations up to 5 ppm, the contents were deoxidized and the medium was inoculated with 4% vol of the inoculum collected during the phase of logarythmic growth of the bacteria (after 24 h). The reaction rate was determined as the degree of sulphates reduction to sulphides measured at certain time intervals.

The apparata and media used in the experiment were sterilized for 20 min at 120°C. The same experiments were performed for blank samples as reference, and the results are means from three measurements. The methodology was designed to measure the effects of the substance tested on microbiological processes, eliminating the influence of chemical processes.

Methods of Analysis

Concentrations of nitrates were measured by the potentiometric method by an ion-selective electrode "Detector".

Concentrations of nitrites were measured spectrophotometrically on a Beckman DU-640 spectrophotometer at λ = 520 nm [7].

Concentrations of sulphides were measured by the iodometric method in CdS precipitate [8].

Concentrations of sulphates were measured by the complexometric method [8].

Concentrations of proteins were measured by the Lowry method using the Foulin reagent. Absorbency was measured at λ = 750 nm [9].

Kinetic equations describing the process of denitrification were obtained using the computer program "Micro-Math Scientist"

Results and Discussion

The results obtained for the process of denitrification run with the *Bacillus licheniformis* bacteria have proved that the threshold of toxicity of APDA introduced into the medium is at its concentration of 10 ppm, Fig 1. When APDA is introduced in lower concentrations - to 5ppm it is responsible for a decrease in the degree of conversion and elongation of the reaction time. At the concentration of 8 ppm a significant inhibition of the process and considerable elongation of the induction period are observed.

Changes in the concentration of nitrites formed as transition products in the process of denitrification [5] are illustrated in Fig. 2. In the first period of the reaction the process of denitrification is significantly inhibited at the stage of nitrites and only after a while are they decomposed to N_2. In the media containing APDA at concentrations higher than 3 ppm, the peaks corresponding to increasing concentrations of nitrites are shifted towards longer times and their height decreases, meaning that the concentration of this transition product decreases. After 170 hours of the reaction (in the media containing from 3 to 8 ppm of APDA) the concentration of nitrites remains at of 50 mg of nitrites nitrogen. At an APDA concentration of 10 ppm, the process of denitrification is fully inhibited; moreover, the microogranisms die, which means that their cells loose the capability of denitrification.

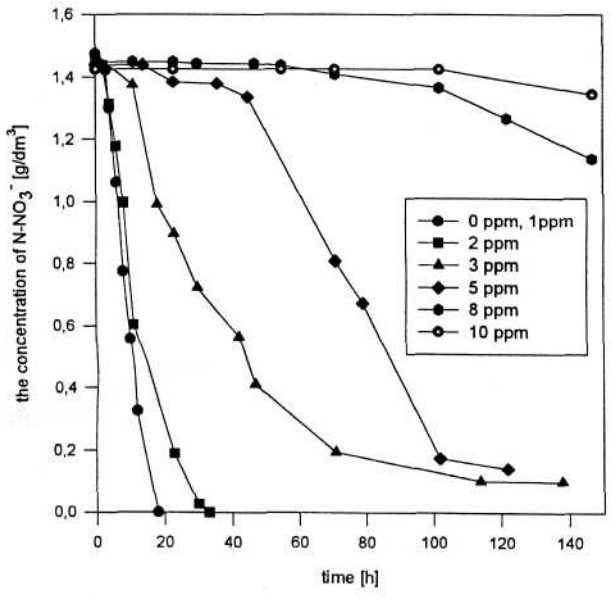

Fig. 1. The effect of increasing concentration of APDA on the process of denitrification run with *Bacillus licheniforniis* bacteria (37°C, pH = 7.5, C/N = 2.33).

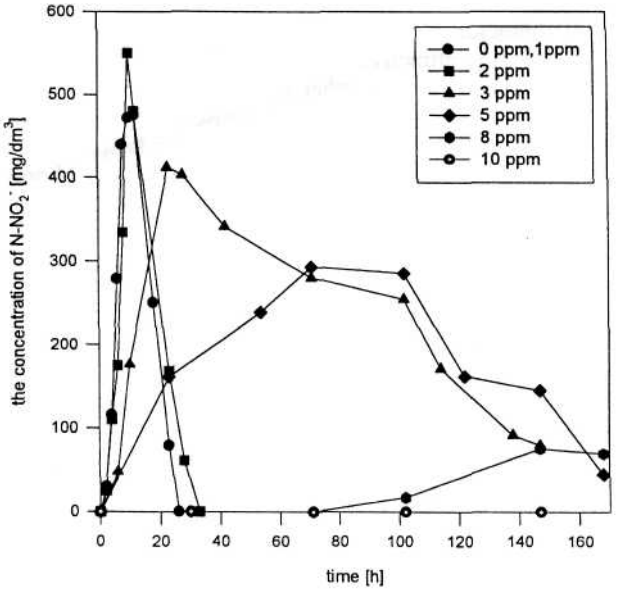

Fig. 2. The effect of increasing concentration of APDA on the formation of nitrite nitrogen in the process of denitrification run with *Bacillus licheniformis* bacteria (37°C, pH = 7.5, C/N = 2.33).

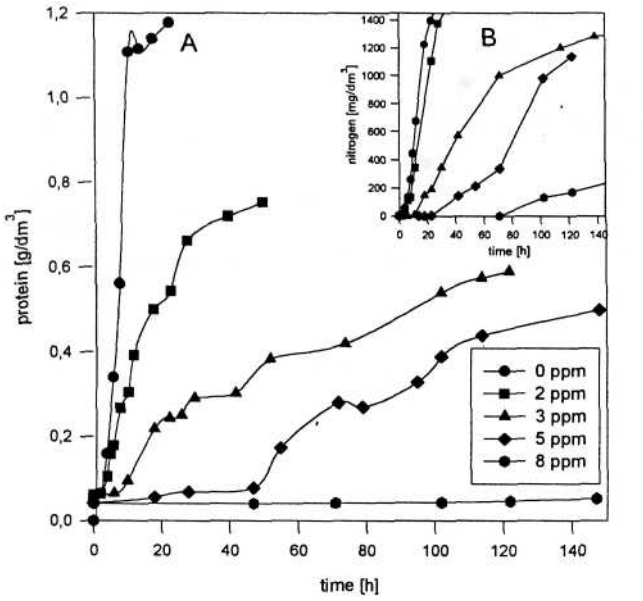

Fig. 3. The effect of increasing concentration of APDA on the accumulation of proteins (A) and nitrogen (B) in the process of denitrification run with *Bacillus licheniformis* bacteria (37°C, pH = 7.5, C/N = 2.33).

Fig. 3 presents the results of the bacteria proliferation accompanying the process of denitrification, controlled by measurements of the protein concentration. Fig. 3B shows the kinetic curves of changes in the concentrations of nitrogen, which is the final product of denitrification. In the control sample free of APDA, the process of bacteria growth in the first few hours of incubation was very intense. With increasing APDA concentration in the medium the increase of the protein concentration is gradually inhibited and the induction period increases. At APDA concentrations of 8 ppm, no increase in the protein concentration was observed, which testifies to the inhibition of the metabolic processes in the bacteria. It is also evident that the increase in the protein concentration corresponding to the growth of the bacteria is correlated with reduction of nitrates. An interesting observation was the enhanced inhibition of the protein concentration growth in the media containing 2.3 and 5 ppm of APDA. These inhibitions correspond to the reaction time intervals of 20-25h, 30-40h and 7O-8Oh. It was established that in these time intervals the transition products - nitrites appearing as a result of reduction of nitrates - undergo decomposition. The observed inhibition of protein concentration increase is related to the adaptation of the microorganisms to the altered medium which contains nitrite reductase - causing decomposition of the transition nitrites [10], and APDA at .different concentrations.

As follows from the analysis of the changes in the concentrations of nitrates, nitrites and proteins (the latter as a result of bacteria proliferation) the process of denitrification can be considered as occurring at the two stages, as in the scheme:

$$\underset{\text{I stage}}{NO_3^-} \xrightarrow{k1} \underset{\text{II stage}}{NO_2^-} \xrightarrow{k2} N_2$$

Attempts were made to describe the kinetics of the process by a few probable models. The model whose predictions were the best correlated with experiment assumes two subsequent irreversible second order reactions. This model is analogous to the earlier provided model of desulfurication [4] and can be described by the following equations:

$$\frac{d[NO_3^-]}{dt} = - k_1 [NO_3^-] [B]$$

$$\frac{d[NO_3^-]}{dt} = - k_1 [NO_3^-] [B] - k_2 [NO_2^-]$$

$$\frac{d[B]}{dt} = Y k_1 [NO_3^-] [B]$$

where:

- $[NO_3^-]$ - is the concentration of N - NO_3^- [g of nitrate nitrogen per dm^3]
- $[NO_2^-]$ - the concentration of N - NO_2^- [g of nitrite nitrogen per dm^3]
- [B] - the concentration of proteins
- k_1 - rate constant of nitrate reduction [$dm^3 g^{-1} h^{-1}$]
- k_2 - rate constant of nitrite reduction [h^{-1}]
- Y - yield coefficient of protein concentration growth.

Table 1. Kinetic parameters of denitrification calculated assuming the model of two subsequent irreversible second order reactions. The process was run with *Bacillus licheniformis* bacteria in media containing different concentrations of APDA (37°C, pH = 7.5, C/N = 2.33).

	Blank sample	2 ppm	3 ppm	5 ppm	8 ppm
t_0 [h]	0	0	0	5	30
k_1 [$dm^3/g \cdot h$]	0.307 ± 0.005	0.31 ± 0.01	0.137 ± 0.008	0.069 ± 0.003	0.013 ± 0.003
k_2 [h^{-1}]	0.221 ± 0.009	0.19 ± 0.02	0.048 ± 0.006	0.043 ± 0.004	0.03 ± 0.02
Y	0.76 ± 0.01	0.51 ± 0.02	0.32 ± 0.02	0.34 ± 0.02	0.24 ± 0.14
Correlation coefficient	0.993	0.989	0.982	0.993	0.999
Standard deviation	0.035	0.066	0.080	0.059	0.032
Square sum	0.0085	0.157	0.251	0.135	0.028
Criteria of the model choice	5.6	3.7	3.0	4.0	5.9

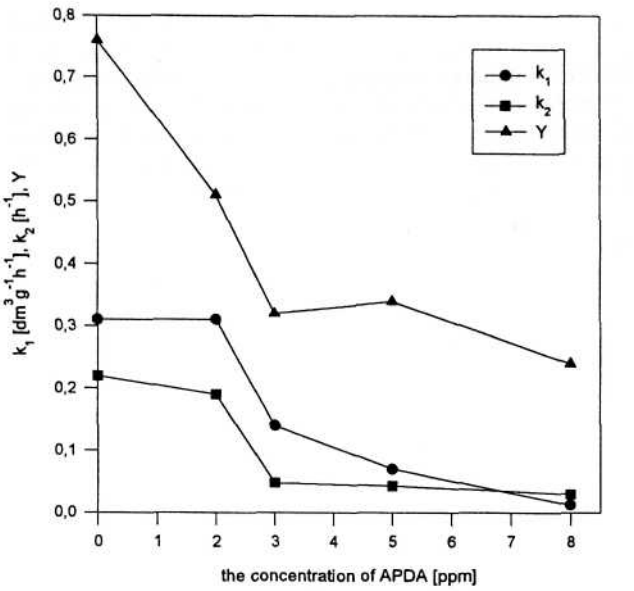

Fig. 4. The rate constants k_1 and k_2 and the yield coefficient of protein concentration growth Y versus the concentration of the APDA introduced into the medium (37°C, C/N - 2.33).

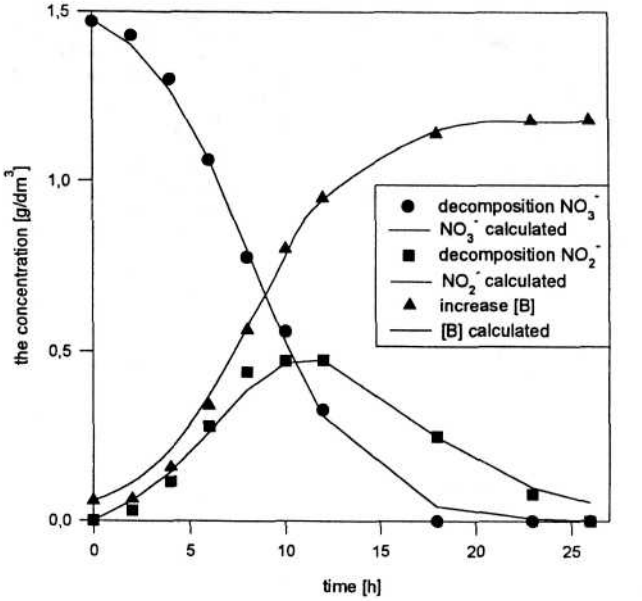

Fig. 5. Correlation of the experimental data (points) with theoretical predictions (lines) assuming the model proposed for denitrification with *Bacillus licheniformis* bacteria in the standard medium (37°C, ph = 7.5, C/N - 2.33).

The kinetic parameters calculated assuming the above model of denitrification with the *Bacillus licheniformis* bacteria in media containing APDA at different concentrations are given in Table 1.

The dependencies of the rate constants kj and k_2, and the yield coefficient of protein concentration growth Y on the concentration of APDA introduced into the media, are displayed in Fig. 4. Statistical analysis of the results has given the correlation coefficient of the curves describing the processes equal to 0.993. This very high correlation confirms that the model assuming two subsequent irreversible second order reactions well approximates the process.

Assuming the above model of denitrification, Figs. 5-7 show the functions describing the process of reduction of NO_3^- NO_2^- and changes in the protein concentration, with the bacteria *Bacillus licheniformis* in the standard medium and in the media containing 2, 3 and 5 ppm of APDA. The compound studied is a strong inhibitor of denitrification and the model can adequately describe the effect of APDA concentrations on the rate of the process.

As far as the process of desulfurication is concerned, the level of APDA toxicity is lower and for APDA concentrations higher than 4 ppm. The process of sulphates reduction is totally inhibited, and the bacteria die. However,

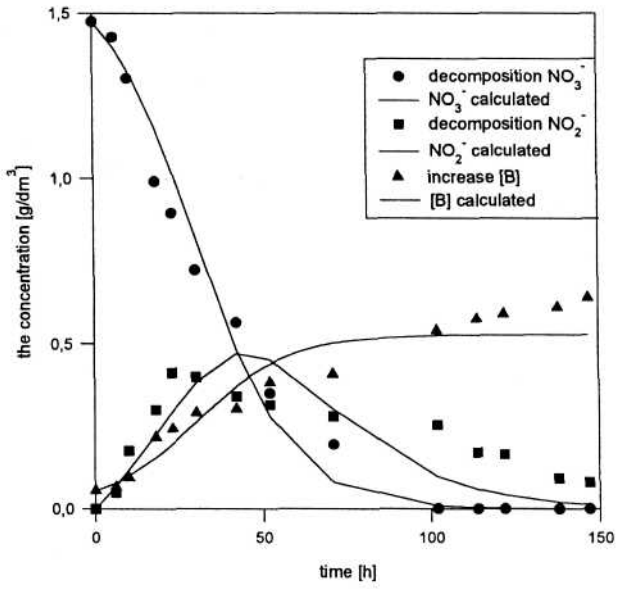

Fig. 6. Correlation of the experimental data (points) with theoretical predictions (lines) assuming the model proposed for denitrification with *Bacillus licheniformis* bacteria in the medium containing 3 ppm of APDA.

Table 2. Microbiological activity of APDA and chloramine T.

Bacteria	Compound	Concentration [ppm]		
		tolerated	inhibiting	toxic
Bacillus licheniformis	APDA	< 2	2-8	> 10
	chloramine T	< 15	15-60	-
Desulfotomaculum ruminis	APDA	< 1	1-3	> 4
	chloramine T	< 210	210-240	> 250

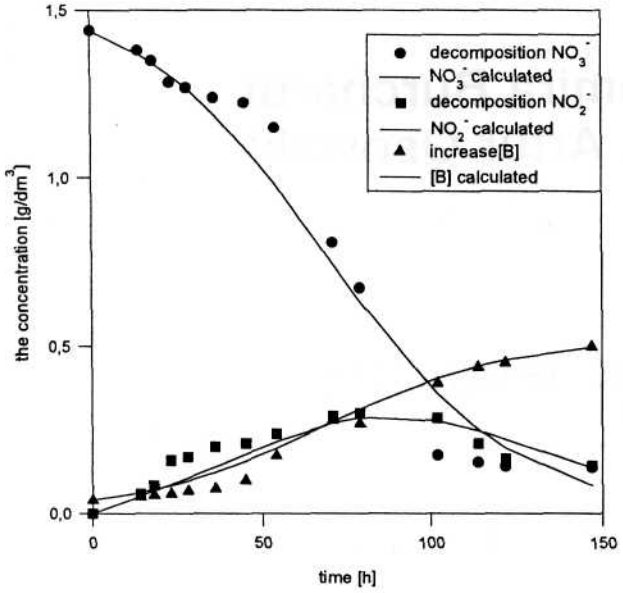

Fig. 7. Correlation of the experimental data (points) with theoretical predictions (lines) assuming the model proposed for denitrification with *Bacillus licheniformis* bacteria in the medium containing 5 ppm of APDA.

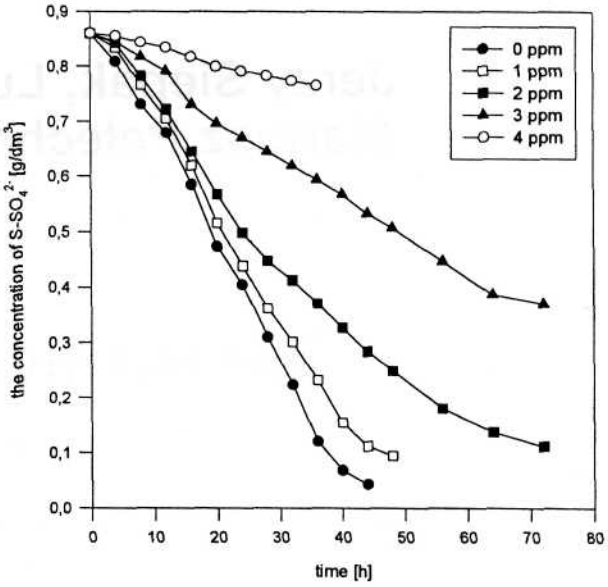

Fig. 8. The effect of increasing concentration of APDA on the process of desulfurication run with *Desulfotomaculum ruminis* bacteria (37°C, pH = 6.8-7.2, C/S = 9.3).

a noticeable inhibition of desulfurication begins already after the introduction of APDA at lppm to the medium (Fig. 8).

As follows from the tests of toxicity performed for *Bacillus licheniformis* and *Desulfotomaculum ruminis* bacteria, APDA is more toxic than chloramine T [11], and the latter bacteria are more sensitive to APDA than the former (Table 2).

The toxicity of APDA is most probably related to its influence on biological cell membranes of the bacteria, in particular on their permeability and potential, which consequently affect the bacteria metabolism.

APDA is soluble in water and can be effectively transported with water over long distances, polluting water and land ecosystems. The results of the tests with the bacteria *Bacillus* and *Desulfotomaculum* are of importance for finding methods for protection against APDA pollution. Further recognition of microbiocide properties of this compound is expected to help develop new effective methods of neutralization.

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