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Original Research

Catabolic Activity of Bacillus Genus Bacteria in DDAO-Containing Media

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Abstract

We studied the effect of N-oxide of N, N-dimethyl-dodecylamine (DDAO) on catabolic activity of the *Bacillus* genus bacteria involved in the process of denitrificantion. The laboratory tests have proved that the presence of DDAO in concentrations greater than 75 ppm extends the time of nitrate (V) reduction, while the concentration of the intermediate-formed nitrates (III) is inhibited at the level of 80 mg/dm^3. Further increase in DDAO concentration, to 10000 ppm, extends the nitrate (V) reduction time to about 115 hours, while the concentration of nitrates (III), formed in the second stage of the reaction, does nor change and remains at a level of 80-100 mg $N\text{-}NO_2^-$/dm^3. The process of denitrification is accompanied by a decrease in COD (biodegradation), whose concentration after completion of the process is 54% in the samples containing 75 ppm DDAO to 31% in the samples containing 10,000 ppm DDAO. Simultaneously, the concentration of proteins decreases from 70% to 30% in the medium containing DDAO in the concentration of 10,000 ppm.

Keywords: *Bacillus licheniformis* bacteria, denitrification, N, N-dimethyldodecylamine oxide (DDAO), proteins, COD

Introduction

N-oxides of lipid amines are frequently used as detergents, of the properties intermediate between amphoteric tensides and non-ionic tensides. The amphoteric properties of derivatives of N-oxides of lipid amines are a result of strong polarization of the N-oxide bond, which means that in the alkaline environment the dominant species has the character of a salt. Such a salt in an acidic solution can attach proton and simultaneously be transformed in the cationic species. Therefore, N-oxides of lipid amines behave similarly to ampholytic tensides [1, 2]. As these amines are usually better tolerated by the human skin and mucous membranes (because of the similarity of their structure and properties to those of proteins), they have been used frequently in the cosmetic industry [3]. To this group of compounds belongs N-oxide of N, N-dimethyldodecylamin (DDAO) of the molecular formula of $C_{12}H_{25}N(CH_3)_2O$ (DDAO), which at intermediate pH properties has cationic and non-ionic molecules at equilibrium [4, 5, 6].

This compound belongs to relatively little known and rarely used surfactants. As follows from literature, it is applied in cosmetics [2] in new means for skin cleaning, for wood conservation, in the substances for removal of inks from plastics [4], in genetic engineering [7, 8] and in the study of protein membranes [9, 10]. The advantage of this group of compounds is a generally strong susceptibility to biodegradation [11] and in solutions they preserve their surface activity in a wide pH range [12].

In view of increasing interest of the industry in this compound, the problems related to utilization of waste, mainly sewage, containing DDAO have become significant. The compound's toxicity should be tested to be able to develop protective measures against its activity. In particular, the effect of DDAO on widespread natural process-

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es of denitrification with the involvement of bacteria from the genus *Bacillus* has been studied [13]. This paper reports results of a study on the effect of different concentrations of DDAO on the kinetics of the process of denitrification and catabolic activity of the *Bacillus licheniformis* bacteria in the medium containing the compound tested.

Materials and Methods

Denitrification — The denitrification bacteria from the genus *Bacillus* were isolated and identified in the way described earlier [14]. The kinetic study was performed at 37°C at pH 8 in closed glass reactors of 20 cm^3 in capacity, containing 10 cm^3 of lactate medium of the composition [g/dm^3]: $N\text{-}NO_3^-$ = 1.4; $Fe(NO_3)_3 \cdot 9H_2O$ = 0.44; NH_4Cl = 0.25; $MgSO_4 \cdot 7H_2O$ = 0.50; $CaCl_2$ = 1,00; $Na_2HPO_4 \cdot 12H_2O$ = 2.50; $C_{org.}$ = 2.52 and microelements [15]. The medium was inoculated with 4% volume inoculum collected after 24 hours of proliferation (logarithmic growth phase). Then a certain amount of the compound tested (DDAO) was added into the reactors chosen from the concentration range 75-10,000 ppm. The rate of the process of denitrification was determined by measuring at certain time intervals the concentrations of nitrates (V), nitrates (III) and proteins from the bacteria biomass. After the process of denitrification in the solutions a level of COD was determined, treated as an arbitrary indication of the content of organic compounds and defined as the amount of oxygen needed for oxidation of organic compounds by chemical reaction. The equipment and media used in the experiments were sterilized at 120°C for 20 minutes in a medical pressure sterillizer. The results were the mean values of three measurements. The reference experiments were performed in the same conditions but without DDAO. In this procedure the effect of DDAO on the microbiological process with elimination of the possible involvement of chemical transformations was studied.

The concentration of nitrates (V) was determined by an ion-selective electrode type DETEKTOR coworking with a chlorsilver reference electrode [16].

The concentration of nitrates (III) was measured spectrophotometrically on a spectrometer DU-640 made by Beckmann, at a wavelength of 520 nm [17].

The concentration of proteins was determined spectrophotometrically by the Lowry method at λ= 750 nm [18].

The content of COD was measured by the dichromate method [19].

Results and Discussion

As follows from the earlier proposed kinetic model of denitrification taking place in a lactate medium and in a medium containing surfactants [14, 15] and bacteria from the genus *Bacillus*, this process is composed of two subsequent irreversible autocatalytic reactions of second order with respect to nitrates (V) and protein, and first order with respect to nitrates (III) and can be described as: $NO_3^- \rightarrow NO_2^- \rightarrow N_2$. That is why the effect of DDAO concentrations on the degree of nitrate conversion and thus on the catabolic activity of the bacteria was determined through measurements of the concentrations of nitrates (V), nitrates (III) and protein coming from the bacteria proliferation at certain time intervals of the process. The results are presented in the form of kinetic curves illustrating the effect of DDAO on the reduction of nitrates (V), nitrates (III) (Figs. 1,2) and increase in the protein concentration (Fig. 4).

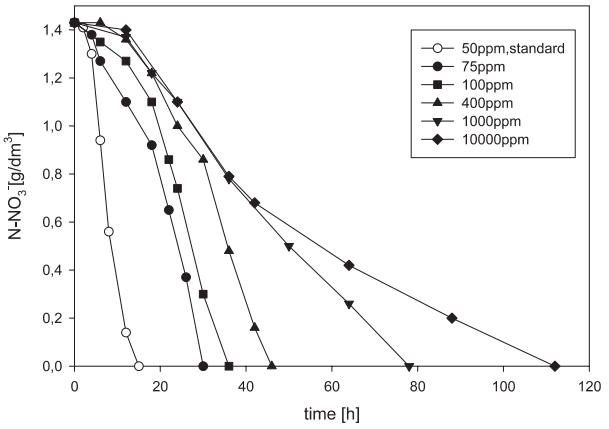

Fig 1. Kinetic curves describing nitrate (V) reduction in a lactate medium with DDAO in different concentrations (37°C, [pH] = 7.5, $[N\text{-}NO_3^-]_0$ = 1.4 g/dm^3, lactate concentration $[C_{org}]_0$ = 10.16 (g/dm^3)

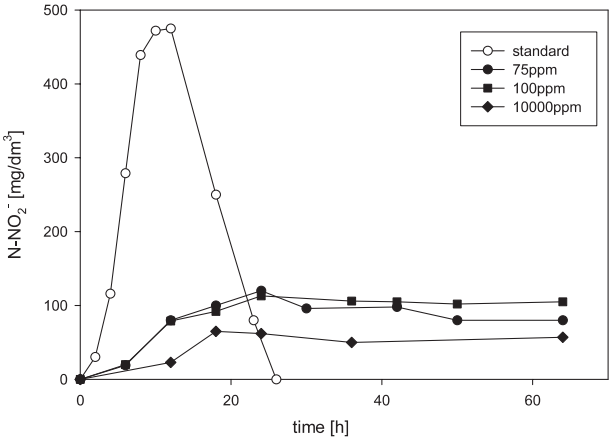

Fig 2. Kinetic curves describing nitrate (III) reduction in a lactate medium with DDAO in different concentrations (37°C, [pH] = 7.5, $[N\text{-}NO_3^-]_0$ = 1.4 g/dm^3, lactate concentration $[C_{org}]_0$ = 10.16 (g/dm^3)

According to our results, only the presence of DDAO in concentrations higher than 75 ppm significantly slows down the reduction of nitrates (V) manifested by almost twice the increased duration of the process relative to the reference sample or experiments with the medium contain-

ing e. g. 50 ppm DDAO (Fig. 1). An interesting observation is a simultaneous inhibition of reduction of nitrates (III) on a level of about 80 mg $N\text{-}NO_2/dm^3$, increasing to 100 mg with increasing DDAO concentration (Fig. 2). In the media free from DDAO (reference samples) the concentration of NO_2- at first increases to about 480 mg $N\text{-}NO_2/dm^3$, which is related to increased ability of the bacteria to produce enzyme catalyzing reduction of the intermediate nitrates (III), and then decreases. After about 25 hours (Fig. 2) the reaction ends with total reduction of NO_2- to free nitrogen.

It should be emphasized that in the whole range of DDAO concentrations studied, nitrates (III) formed as intermediates at the second stage of denitrification are not fully reduced to nitrogen. The process is inhibited at a level of 50-100 mg $N\text{-}NO_2/dm^3$ at a simultaneous total reduction of nitrates (V) taking place in a time dependent on the concentration of DDAO in the medium (Fig. 1). The presence of DDAO in the medium in a concentration of 100 ppm causes a further increase in the time of nitrate (V) reduction, to about 38 hours and growing to about 115 hours at the DDAO concentration of 10,000 ppm. The effect of DDAO in the concentration range studied on the time needed to transform nitrates (V) is illustrated in Fig. 3.

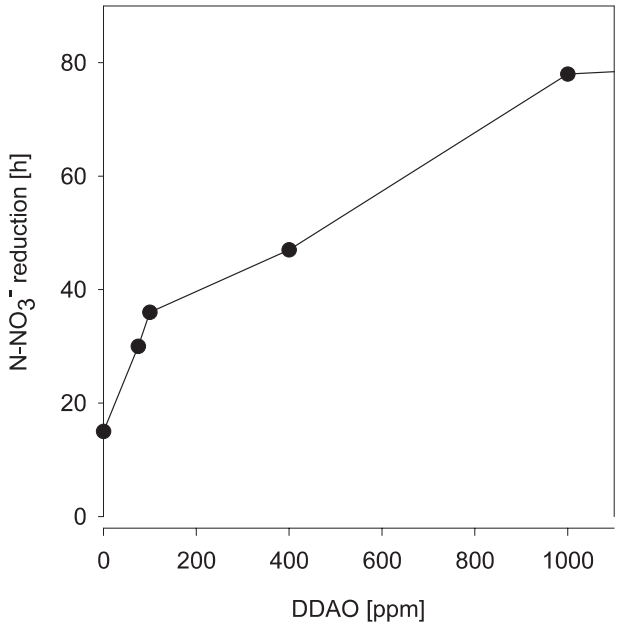

Fig. 3. The effect of DDAO concentration on the duration of nitrate (V) reduction taking place with involvement of the bacteria strain *Bacillus licheniformis* (37°C, pH = 7.5).

The results indicate that the presence of DDAO on the one hand slows down the first stage of nitrate (V) reduction, which takes place at 100% depending on the DDAO concentration, while on the other hand it inhibits the second stage of the reduction at a level of the intermediate nitrates (III) at a concentration of about 100 mg $N\text{-}NO_2$. Nitrates (III) are more toxic than nitrates (V). In the environmental conditions this means that the bacteria are not able to reduce nitrates (III) formed as intermediates in the media with DDAO in concentrations higher than 75 ppm, and the concentration of these nitrates remains at a level of about 100 mg $N\text{-}NO_2/dm^3$ (Fig. 2).

The changes in the concentration of proteins caused by the proliferation of the bacteria during the process of denitrification for different DDAO concentrations are shown in Fig. 4.

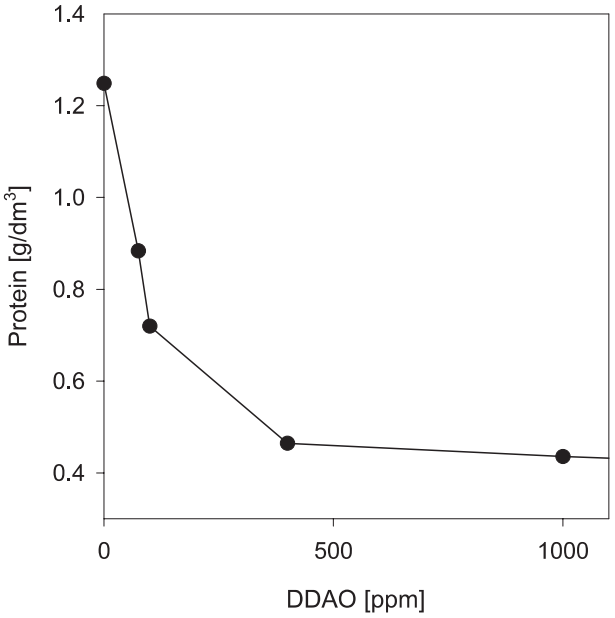

Fig. 4. The concentrations of proteins measured after the process of nitrate (V) reduction taking place with involvement of *Bacillus licheniformis* bacteria in the media containing DDAO in different concentrations.

The greatest decrease in the protein concentration, especially in the media containing up to 100 ppm DDAO, is correlated with the increasing time of the process of denitrification (Fig. 3). Further decrease in the level of proteins at higher concentrations of DDAO, from 58% to 37%, is most probably related to the level of concentration of unreduced nitrates (III), whose presence does not facilitate the adaptation of the bacteria to the altered medium. On the basis of the tests performed in the media without lactate it can be concluded that the bacteria have not developed the ability to use DDAO as a source of coal and energy, and the compound tested only causes inhibition of the bacteria growth and an increase in the time of nitrate (V) reduction.

In this context the changes in COD of the medium after the reaction the unreacted organic substrates, metabolites and micro-organisms (protein), are fully understood. The decrease in COD (%) after denitrification in the media containing DDAO in concentrations up to about 400 ppm is at a level of 50% (Fig. 5). In the media containing higher concentrations of DDAO — up to 10,000 ppm — the reduction of COD is only 31.2%.

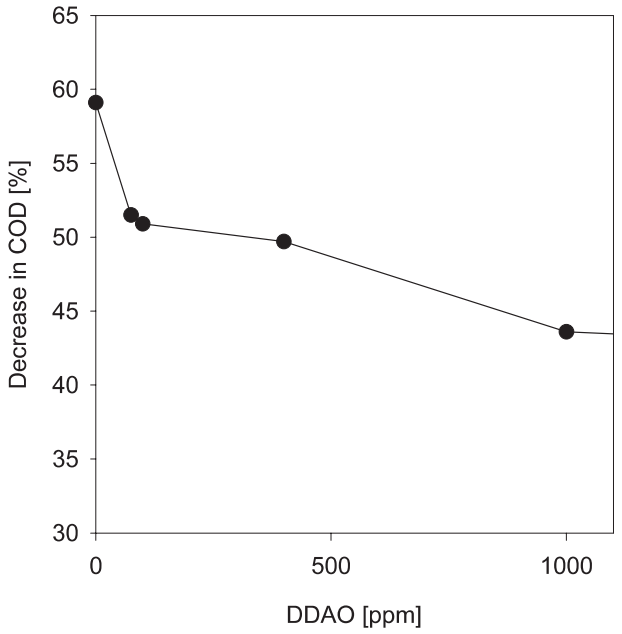

Fig. 5. Changes in COD [%] after the process of nitrate (V) reduction taking place with involvement of *Bacillus licheniformis* in a medium containing DDAO.

It has been shown that DDAO in concentrations up to 50 ppm has no effect on the process of denitrification. The kinetic curves obtained in such experiments coincide with the curve recorded for the reference sample (Fig 1). With DDAO concentration increasing from 75 ppm to 100 ppm and 400 ppm, the time needed for total decomposition of NO_3^- to N_2 quickly increases. With a further increase in DDAO concentration, this time still increases but much slower and for the DDAO concentration of 10,000 ppm it is 115 hours. In the whole DDAO concentration range studied the second stage of denitrification is inhibited as a level of about 100 mg $N\text{-}NO_2\text{-}/dm^3$, which means that the undecomposed toxic nitrates (III) are left in the medium. This result points to a possible way in which toxic substances can get into the natural environment, while according to the standards they cannot be introduced into surface waters [20]. It is not excluded that the reason for the situation is that the bacteria had too little time to produce enzymes, allowing decomposition of nitrates (III) to free nitrogen. This finding indicates that a similar situation can happen for other toxic chemicals, which may get into the natural environment at different stages of processes taking place in the environment. In view of the above, determination of the rate and range of microbiological transformations, their stages and possible metabolites is of importance for predicting the level of pollution necessary to establish the strategy of sustainable development. In each individual case this requires a series of experiments postulated already by K. Mikschta [21], aimed at determination of toxicity and susceptibility to biological decomposition of new chemical compounds introduced into the natural environment.

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