Polish Journal of Environmental Studies Vol. 6, No. 4 (1997), 5–8

Original Research

The Influence of DDDM on Denitrification and Desulfurication Processes

B. Brycki, K. Szymańska, K. Seifert, F. Domka

Faculty of Chemistry,
Adam Mickiewicz University,
60-780 Poznań, ul. Grunwaldzka 6, Poland

Received 25 November, 1997
Accepted 10 February, 1997

Abstract

The influence of didecyldimethylammonium chloride (DDDM) on the processes of denitrification - occurring with a contribution of *Bacillus licheniformis* bacteria, and desulfurication - with a contribution of *Desulfotomaculum ruminis* bacteria, was studied. It has been shown in laboratory conditions that the compound tested is not assimilable by the cultures of the above mentioned heterotrophic bacteria and the values of toxic concentrations of DDDM for the studied microorganisms were determined.

Keywords: *Bacillus licheniformis*, *Desulfotomaculum ruminis* bacteria, biodegradation, toxicity, denitrification, desulfurication, didecyldimethylammonium chloride.

Introduction

Multifunctional character of tertiary ammonium compounds makes them important components in many technological processes and many materials of everyday household use [1, 2]. Depending on the number of carbon atoms of organic molecules attached to the tertiary nitrogen forming the cation, they can be used as washing means (surfactants) and disinfectants [3, 4]. After use they can penetrate into the natural environment in unaltered form and cause harmful effects, for example when at concentrations higher than the threshold of toxicity their presence can inhibit the self-purification process and delay metabolic processes.

Didecyldimethylammonium chloride (DDDM) has been widely used as an active component of various fungicidal, algaeicidal and bactericidal agents [5, 6]. Used for cleaning and disinfection of various devices, machines and apparatuses in the food industry, medicine and sanitary product maintenance [7, 8]. According to literature, the spectrum and effectiveness of DDDM activity depends on its concentration, type of microorganism, time of contact, pH of the environment, temperature and chemical composition of the support [9]. The widespread use and toxicity of DDDM has prompted studies on its biodegradation and the degree of harmful effects it has on the environment, in order to find the right means of environmental protection. In particular, we were interested in the effects of DDDM on the processes of denitrification and desulfurication occurring in nature with participation of nitrate and sulfate reducing bacteria [10, 11]. Realising a possible threat to ground table waters and soils, and consequences of the above, it is necessary to establish the threshold admissible concentration of DDDM which would not give harmful effects to microorganisms in the natural environment.

This paper reports results of the study on the DDDM effect on the kinetics of growth of *Bacillus* bacteria responsible for denitrification and *Desulfotomaculum ruminis* bacteria which reduce sulfates and degrades organic compounds to obtain energy and growth substrates for their metabolism.

Material and Methods

Denitrification

Bacteria from the *Bacillus* genus controlling denitrification were isolated and identified in the procedures described earlier [12]. Kinetic studies were conducted at 37^{0}C in air tight glass reactors of 20 cm^3 in volume. The reactors were filled with 10 cm^3 of lactate medium [12] to which 4% volume of an inoculum obtained after 24 hours of bacteria growth (the phase of logarithmic

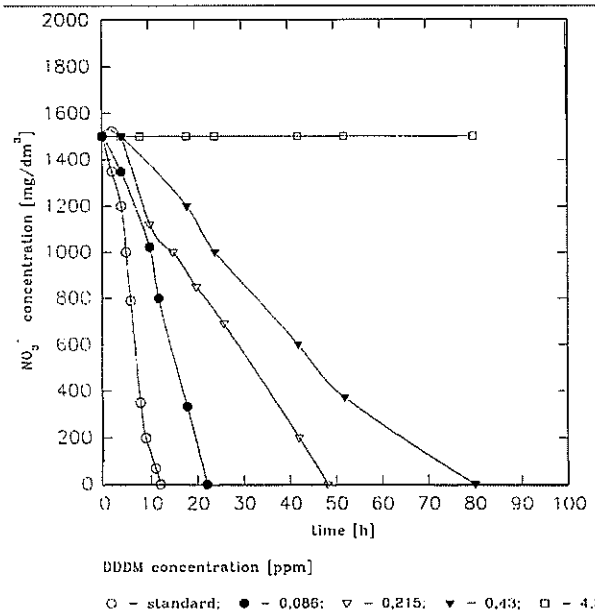


Fig. 1. The effect of DDDM concentration on microbiological reduction of nitrates with *Bacillus licheniformis* bacteria (T=37°C, pH=8)

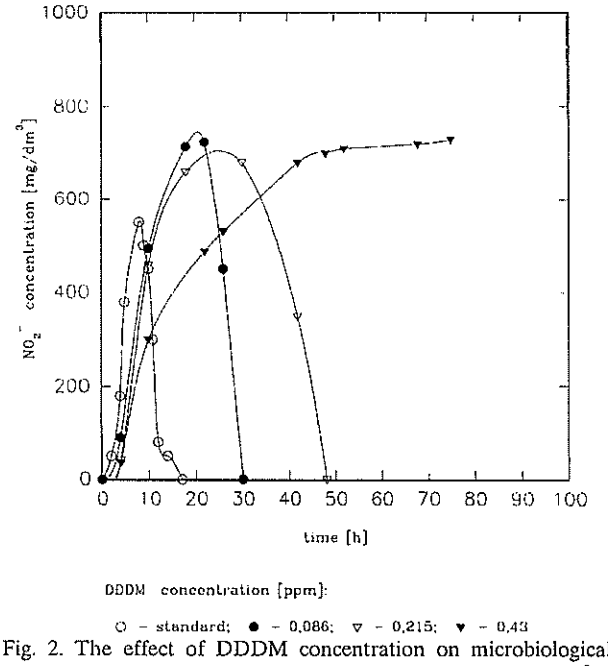


Fig. 2. The effect of DDDM concentration on microbiological reduction of nitrates with *Bacillus licheniformis* bacteria (T=37°C, pH=8)

growth), was introduced. The process was performed at pH 8. The rate of denitrification was measured at certain time intervals by determining the concentration of nitrates and nitrites. The tested DDDM was added into the reactors in concentrations of 4.3, 0.43, 0.215, 0.086 ppm. The concentration of nitrates was measured potentiometrically using an ion-selective electrode DETECTOR, while that of nitrites by the measurement of absorption of a dye composed of nitrates, sulfanilamide and N-1-naphtylethylene chloride in the presence of phosphoric acid [13].

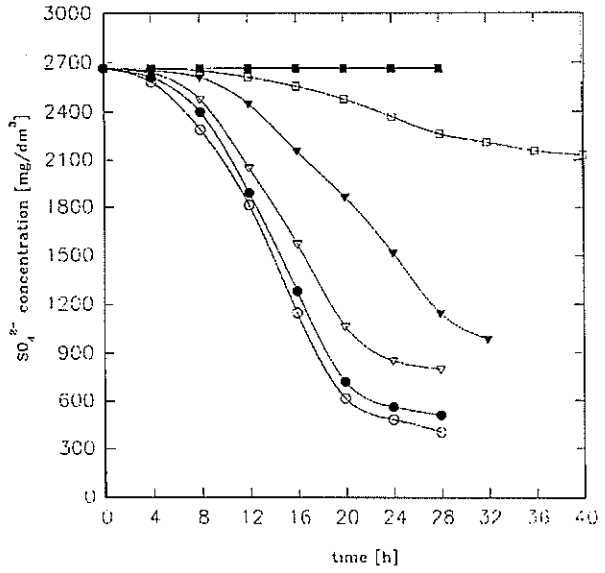


Fig. 3. The effect of DDDM concentration on dissimilatory reduction of sulphates with *Desulfotomaculum ruminis* bacteria (T=37°C, C/S=3.6)

Desulfurication

The bacteria reducing sulfates were isolated and identified as *Desulfotomaculum ruminis* according to the earlier described procedure [14]. The kinetic studies were conducted in glass reactors containing 15 cm^3 of the culture medium [14] inoculated with 4% volume of the inoculum taken each time from the pure bacteria culture after 24 hours of growth. The process was conducted at 37°C, and medium pH 7. The concentrations of the tested DDDM introduced into the medium with the bacteria were: 11.0, 21.5, 25.8, 30.1, 32.3 ppm. The rate of the reaction was determined as the degree of the microbiological reduction of sulfates to sulfides measured at certain time intervals. In order to do this the reactors were blown with helium and the reduced hydrogen sulfide was absorbed in a bulb containing 0.02 M solution of cadmium acetate, and the sulfides (CdS) concentration was determined iodometrically.

In the studies of both denitrification and desulfurication measurements were also performed on the control samples without DDDM, and the reported results are averages from 3 experiments.

Results and Discussion

As follows from our results, from the two kinds of bacteria studied, the *Bacillus* ones (responsible for denitrification) are much more sensitive to the presence of DDDM than *Desulfotomaculum* taking part in desulfurication. The influence of DDDM on the proc-

ess of denitrification is illustrated in Figure 1. Denitrification is a multistage redox process in which nitrates are the last link in transportation of electrons during oxidation of organic compounds, and it is catalyzed by enzymes from the group of reductases which are found in cells of the studied denitrificators. This process involves two subsequent 1st order reactions [15], according to the scheme:

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2$$

The results show that for kinetic curves illustrating the rate of denitrification obtained for the reference samples and samples containing DDDM at low concentrations have a similar shape typical of enzymatic reactions [16]. The rate of bacteria growth determined as the temporary changes of nitrate concentration in the reference samples and samples with a low concentration of DDDM depends on the rate of consumption of the limiting substrate (lactate) and on the concentration of DDDM. The total inhibition of the process takes place at the DDDM concentration of 4.3 ppm. Tests performed for samples with the media which did not contain the lactate proved that DDDM resists the attacks of the bacteria, i. e. it is not assimilable by them, therefore the total inhibition of denitrification in the presence of DDDM at 4.3 ppm must be due to its toxic influence on the bacteria. The presence of DDDM at lower concentrations results in a significant elongation of the time of the process (see Table 1).

Fig. 2 presents results of the study on the effect of DDDM at different concentrations on reduction of nitrites which are the intermediate products of denitrification [15].

The results show that although the reduction of nitrates in the presence of DDDM at the concentration of 0.43 ppm occurs only after 80 hours, the whole process is inhibited at the stage of formation of nitrites [17] known to be highly toxic. In view of this it should be regarded that the presence of DDDM at concentrations admissible from the point of view of nitrate reduction may still be harmful to the environment because it stops denitrification at the stage of nitrite formation. Therefore, only the presence of DDDM in concentrations lower than 0.4 ppm can be concluded as not toxic to microorganisms responsible for the process of denitrification, an important element in the cycle of elimination of nitrogen compounds from waters and wastes. The only manifestation of the toxic effect of this compound when at lower concentrations is a noticeable slow down in the reaction rate.

The effect of DDDM on *Desulfotomaculum* bacteria responsible for the process of desulfurication is illustrated in Fig. 3.

As follows from the results of this series of experiments, the *Desulfotomaculum ruminis* bacteria are about 7.5 times less sensitive to the toxic influence of DDDM than the bacteria from the *Bacillus* genus. The threshold toxic concentration of this compound in this case is 32.3 ppm. In the presence of DDDM at a concentration of 11 ppm in the medium the rate of desulfurication is practically the same as in the reference samples.

According to literature data [18] the concentration of DDDM toxic for hetrotrophic *Desulfovibrio desulfuricans* bacteria (which are also involved in the process of desulfurication) is as low as 7.5 ppm. Such a low value of the lethal DDDM concentration in this case may be related to the fact that this species of bacteria does not go through a sporulation phase and the mechanism of desulfurication it takes part in is different [19].

As follows from the results of our study, the toxic effect of DDDM is much more pronounced in the processes of denitrification than in those of desulfurication. Moreover, it should be remembered that lethal concentrations of DDDM can be much different for different species of bacteria, and that the known phenomenon of adaptation of bacteria to extreme conditions can result in a shifting of these values. Very often bacteria cells do not use the energy for enzyme production before it is required to perform a special task like, for example, a catalytic degradation of DDDM. Thus, enzyme production can be imposed by DDDM or an appropriate product of transformation as has been shown by the results reported by Van Eyk and Bartels [20]. The observed difficult biotransformation of DDDM can also be a consequence of molecular structure of the compound, as according to literature data compounds whose molecules are made up of a large number of chains are difficult to metabolize. Pirnik et al.[21] suggest that metabolism of such branched organic compounds is also inhibited by the products of biotransformation.

As follows from our studies, DDDM is assimilable neither by *Bacillus licheniformis* nor *Desulfotomaculum ruminis* bacteria. In the medium containing DDDM as the only source of carbon, neither of the two above mentioned kinds of bacteria grew, meaning that DDDM did not undergo biodegradation in these conditions. Moreover, DDDM was found to inhibit denitrification and desulfurication already when present in low concentrations.

The tests of toxicity carried out for *Bacillus licheniformis* bacteria have shown that reduction of nitrates is inhibited at a DDDM concentration of 4.3 ppm, while reduction of nitrites occurs at 0.43 ppm. At DDDM present in lower concentrations the reduction of nitrates to nitrogen is not broken at the stage of nitrite formation.

The tests of DDDM toxicity performed for *Desulfotomaculum ruminis* have shown that the process of desulfurication is totally inhibited at DDDM concentrations of 32.3 ppm.

A general conclusion following from the results of our studies is that DDDM will be harmful for the processes of denitrification and desulfurication in natural environment only if present at concentrations higher than the determined toxic ones.

References

1. KOURAI H., et al. The antimicrobial characteristics of quaternary ammonium salts. Part XI. Quantitalive Structure - activity relationship of antimicrobial n-laurylpyridinium iodided. J. Antibact. Antifung. Agents, **13**, 245, **1985**.
2. JUNGERMANN E., Cationic Surfactents, Ed. M. Dekker, INC, New York, **1970**.
3. REENE D.F., PETROCCI A.N. Formulating quaternary cleaner disintectants to meet EPA requirements. Soap Cosmet. Chem. Spec., **8**, 33, **1980**.
4. STARK R.L., Aqueous antimicrobial ophthalmic solutions. U.S. Patent **4**, 525, **346**.
5. PETROCCI A.N., GREEN H.A., MERIANOS J.J., LIKE B., The Properties of dialkyldimethyl quaternary ammonium compounds. C.S.M.A. Proceedings of the 60th Mid-Year Meeting, May **1974**, pp. 87-89. Firma IODEX S.A., Poznań.
6. GENNARO A.R. (ed.), Antimicrobial drugs. In Remington's Pharmaceutical Sciences, 17th edition. Easton, PA, Mack Publishing, pp. 1158-1169, **1985**.
7. BERNARDUCCI E., HARRISON K.A., Isotropic laundry detergents containing polymeric quaternary ammonium salts. U.S. Patent **4,755,327**.
8. FRANKLIN T.J. SNOW G.A., Biochemistry of Antimicrobial Action. 4th Edition, London, Chapman and Hall, **1989**.
9. MOŻEJEW E.A. Zagrożienia wodojemow powierchnostwo-aktiwnymi wieszczestwami. Izdat, Medicina, Moskwa, **1976**. Determination of Cationic Active Maller Content in Bardac-22 (Direct two-phase titration procedure). International Standard ISO 2871 (Ed. **1988**)
10. JUSZCZAK A., DOMKA F. Denitryfikacja jako proces katalizy enzymatycznej i jej zastosowanie w technologii oczyszczania ścieków azotanowych. Wiad. Chem., **42**, 261, **1988**.
11. DOMAGAŁA Z., DOMKA F. Desulfurikacja i jej niektóre aspekty ekologiczne. Wiad. Chem., **48**, 105, **1994**.
12. DANECKA-BIAŁEK M., DOMKA F. The Effect of Ammonia Ions and Oxygen on the Kinetics of Denitrification and Growth of Bacillus Licheniformis Bacteria. Polish Journal of Envir. Stud., **3**, 21, **1994**.
13. MARCZENKO Z. Kolorymetryczne oznaczanie pierwiastków, WNT, W-wa, **1968**.
14. DOMAGAŁA Z., DOMKA F. Estimation of the Effect of Desulfotomaculum Ruminis Bacteria on the Process of Degradation of Simple Organic Substrates. Envir. Prot. Eng., **17** (3-4), 83, **1991**.
15. JUSZCZAK A., DOMKA F. Badania nad kinetycznym modelem procesu biodenitryfikacji. Chemia Stos., **XXXII** (2), 301, **1988**.
16. KALETA K. Wyznaczanie parametrów kinetycznych reakcji enzymatycznej przy użyciu scałkowanej postaci równania szybkości reakcji. Post. Bioch. **25**, 119, **1979**.
17. CHRISTENSEN M.H., HARREMOES P., Progress in Water Technology, **2**, 509, **1977**.
18. Biotech Control Laboratories Inc., Waterford, N.Y. Report BCI-2670 of August 25, **1970**, API-38 tests BARDAC-22.
19. KHAUER R.K., BADZIONG W. Dissimilatory Sulfate Reduction. Energetic Aspects. Biology of Inorganic Nitrogen and Sulfur. Ed. H.Bothe and A.Trebst, Springer-Verlag, Berlin, Heidelberg, New York, **1981**, pp. 188.
20. van EYK, BARTELS T.J. Introduction of Paraffin Oxidation. Paraffin Oxidation in Pseudomonas aeruginosa. Journ. Bacter., **96**, 706, **1968**.
21. PIRNIK P.M., ATLAS R.M., BARTHA R., Hydrocarbon Metabolism by Brevibacterium erythrogenes: Normal and Branched Alkanes. Journ. Bacter. **119**, 868, **1974**.