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The Effect of Oxidizing Biocides on Desulfurication and Denitrification Processes

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

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

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Abstract

The effect of oxidizing microbiocides (DCDMH and BCDMH) on the activity of bacteria in the processes of denitrification *(Bacillus licheniformis)* and desulfurication *(Desulfotomaculum ruminis)* is studied.

Desulfotomaculum ruminis bacteria have been found to be more resistant than *Bacillus licheniformis* bacteria to the inhibiting effect of active halogen released from the studied halogenodimethylhydantoines.

The study has shown that the compounds tested can be used as a safe source of slowly released halogen for disinfection.

Keywords: bacteria, *Bacillus licheniformis, Desulfotomaculum ruminis,* toxicity, denitrification, desulfurication, DCDMH (dichlorodimethylhydantoine), BCDMH (bromochlorodimethylhydantoine), indigo carmine.

Introduction

Dichlorodimethylhydantoine (DCDMH) and bromochlorodimethylhydantoine (BCDMH) have recently enjoyed much interest as oxidizing biocides and safe alternatives of gaseous chlorine in many processes of the chemical industry [1, 2, 3, 4]. As follows from rich literature and numerous patent applications, these compounds have been commonly used, either by themselves or as components of different agents, for water purification (disinfection of swimming pools and closed water systems) [5, 6], for removal of mud and sludge from sewage waste [7], for reduction of emission of sulphur compounds and air protection against hydrogen sulfide [{$], in cleaning and disinfecting agents used in the home and industry [9]. They are also used as components of new bleaching agents as they do not destroy fabrics [10] and do not corrode metal [11, 12].

Halogen derivatives of dimethylhydatoine, which are the source of halogen, occur in the solid state form and chlorine or chlorine and bromine are released in the process of hydrolysis, depending on environmental conditions. This solution ensures a low level of free halogen in the environment and minimises metal corrosion, which may be even totally eliminated by the effect of other components. The main advantage of these substances is that they are a convenient source of active halogen occurring in a chemically stable and easy to use form.

A widespread use of BCDMH and DCDMH prompted us to check the effect of these compounds on the processes of denitrification and desulfurication closely related to the cycles of conversion of sulphur and nitrogen.

The main aim of the study was to recognise their toxicity in order to protect the environment. This paper reports the effect of the presence of these compounds in different concentrations on the process of denitrification taking place with *Bacillus licheniformis,* and desulfurication taking place with *Desulfotomaculum ruminis* bacteria.

Corespondence to: Domka F. tel. 0-61 829-13-42

Materials and Methods

Denitrification. The bacteria from the genus *Bacillus* taking part in the process of denitrification were isolated and identified in the way described in [13].

Kinetic study was performed at 37°C, pH 1.5, in sealed glass reactors of 20 cm^3 filled with 10 cm^3 of lactate medium of the following composition [g/dm^3]: N_{No3-} = 1.40, $Fe(NO_3)_3 \cdot 9H_2O$ = 0.44, NH_4Cl = 0.25, $MgSO_4 \cdot 7 H_2O$ = 0.50, $CaCl_2$ = 1.00, $Na_2HPO_4 \cdot 12H_2O$ = 2.50, sodium lactate = 2.52 (16 cnWdmj medium) and microelements [13]. The medium was inoculated with 4%v of the inoculum collected after 24 hours of growth (the phase of logarithmic growth) and then, a certain amount of the compounds studied: BCDMH or DCDMH, in different concentrations up to 200 ppm, was added. The rate of denitrification was determined by periodical measurements of the concentration of nitrates and nitrites.

Desulfurication. The bacteria reducing sulphates were isolated and identified as *Desulfotomaculum ruminis* by the method described in [14].

Kinetic study was performed at 37°C under helium (in anaerobic conditions) at pH from 6.8 to 7.2, in sealed glass reactors containing 50 cm^3 of sterilized modified Starkey medium of the composition [g/dm^3]: $MgSO_4 \cdot 7H_2O$ = 2.00, Na_2SO_4 = 2.66, NH_4C1 = 1.00, K_2HPO_4 = 5.00, $CaCl_2$ = 0.13, Mohr salt = 0.006, sodium lactate = 25.00 and microelements [14]. The medium was inoculated with 4%v of the inoculum collected after 24 hours of bacteria growth (logarithmic growth phase). The compounds tested (BCDMH and DCDMH) were added in different concentrations up to 300 and 400 ppm, respectively. The reaction rate was determined by the periodically measured amount of sulphides to which sulphates were reduced.

The instruments and media used in the experiment were sterilized for 20 min at 120°C. In the same conditions parallel experiments were carried out on the reference samples (without the compounds tested). The data given in Table 2 and Figs. 2 - 4 are the averaged results of three experiments. This procedure allowed measurement of the effects of the compounds studied on the microbiological process with the chemical processes disregarded.

Methods of Analysis

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

Concentrations of nitrites were determined spectrophotometrically on a Beckman DU-640 spectrometer at λ = 520 nm [15].

The concentration of sulphides was found in the precipitated CdS by the iodometric method [16].

The concentration of sulphates was determined by the complexometric method [16].

Results and Discussion

The main advantage of the halogen derivatives of tested dimethylhydantoine (BCDMH and DCDMH) is the fact that they are the source of halogen in the active form of HClO and ClO^- or HBrO and BrO^- in a chemically stable, safe and easy to use form. The effectiveness of the influence of these oxidizing biocides on microorganisms depends on their concentration and environmental conditions, similarly as in the case of earlier studied TCICA [17].

BCDMH and DCDMH are solid substances hardly water-soluble and as a result of hydrolysis provide halogen at a level necessary for oxidation and disinfection. The rate of halogen release depends on the rate of hydrolysis and release of CIO^- or CIO^- and BrO^- ions. Table 1 presents chemical characteristics of these compounds: solubility, pH of water solutions, percent concentration of bromine and chlorine in BCDMH and chlorine in DCDMH.

As follows from the test of chemical activity of the compounds, in which the time of indigo carmine oxidation was assumed as an indicator of changes in the environment, BCDMH is about 26 times more active than DCDMH. In the test discoloration of indigo carmine was performed with only 0.3 g of BCDMH and as much as 8 g of DCDMH, see Table 1. This result testifies to a much different chemical activity of these preparations. The relationship between the concentration of BCDMH or DCDMH and the time needed for discoloration of indigo carmine is shown in Fig. **1.**

Table 1. Physical and chemical properties of BCDMH and DCDMH (25°C).

No.	Property	BCDMH	DCDMH
1	Solubility in H_2O [g/dm^3]	0.25	0.21
2	pH of water solutions	4.03	4.05
3	Halogen content [%]	33.13 (Br) 14.70 (Cl)	36.04(Cl)
4	Amount of the compound needed for total discoloration of indygocarmin (0.03 g/100 cm^3) [g]	0.3	8
5	Concentrations toxic against: *Bacillus licheniformis* [ppm] converted into the amount of halogen: *Desulfotomaculum ruminis* [ppm] converted into the amount of halogen [ppm]	250 37 (Cl); 83 (Br) 400 59 (Cl); 132 (Br)	200 71 (Cl) 300 110 (Cl)

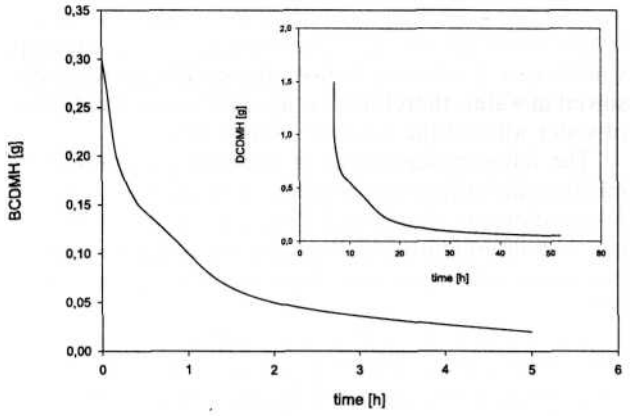


Fig. 1. The effect of concentration of BCDMH and DCDMH on time needed for discoloration of indigo carmine.

Biological activities of BCDMH and DCDMH were tested in the process of dissimilative reduction of sulphates (desulfurication) and dissimilative reduction of nitrates (denitrification) taking place with the involvement of the bacteria *Desulfotomaculum ruminis* and *Bacillus licheniformis*, respectively. Fig. 2 presents results of a study of the influence of these compounds on *Desulfotomaculum ruminis* bacteria in the process of desulfurication. With increasing concentration of BCDMH and DCDMH in the medium, a systematic decrease in the reaction rate is observed, testifying to a decreasing activity of the bacteria as a result of the inhibitory effect of these biocides. The difference in the course of the kinetic curves is that in the case of BCDMH an increase in its concentration to 200 ppm causes a proportional increase in the inhibition, while in the case of DCDMH an increase in its concentration to 70 ppm causes an insignificant inhibition of the process and only above this level an increase in the concentration of this compounds has a strong inhibitory effect. The bactericidal effect is observed for BCDMH at a concentration of 400 ppm and for DCDMH at 300 ppm, in both cases at these concentrations the process of desulfurication is irreversibly inhibited.

The *Bacillus licheniformis* bacteria used in the process of denitrification are much more sensitive to the influence of BCDMH and DCDMH than *Desulfotomaculum ruminis* ones, Fig. 3. As follows from the kinetic curves of the process of denitrification, for BCDMH in concentrations increasing up to 100 ppm, an increase in the inhibition of the process is proportional. The concentration of 200 ppm is toxic for the bacteria studied and at this concentration the process is significantly inhibited. At the same time a transient increase in the concentration of toxic nitrites is observed and they undergo decomposition after a relatively long time.

DCDMH introduced into the medium also causes inhibition of denitrification; however, the inhibition is observed only after 60 hours and is little dependent on DCDMH concentrations in the range 25-100 ppm. The total and irreversible inhibition of denitrification occurs at the DCDMH concentration of 200 ppm. In this reaction, the level of the toxic nitrites formed in the process

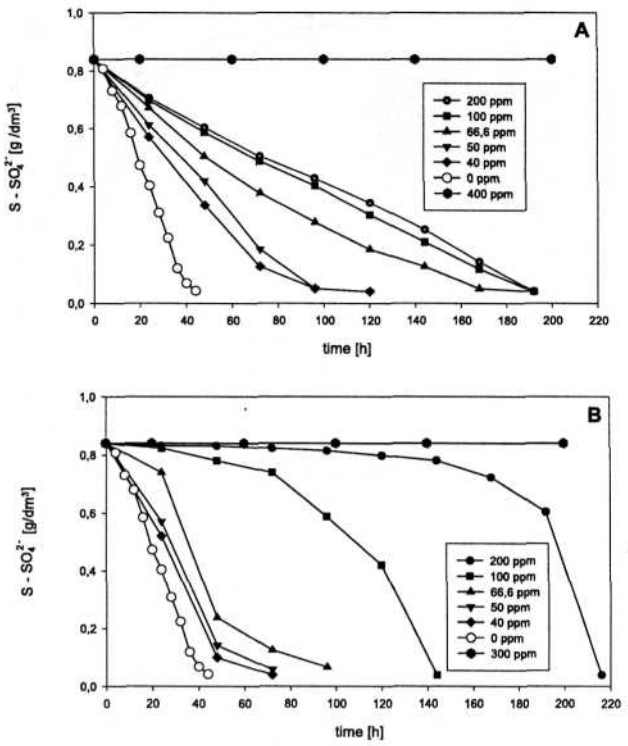


Fig. 2. The effect of l-bromo-3-chloro-5,5-dimethylhydantoine (A) and l,3-dichloro-5,5-dimethylhydantoine (B) on desulfurication process with *Desulfotomaculum ruminis* bacteria (37°C; pH = 6.8-7.2; C/S = 9.3; BCDMH and DCDMH - solid substance).

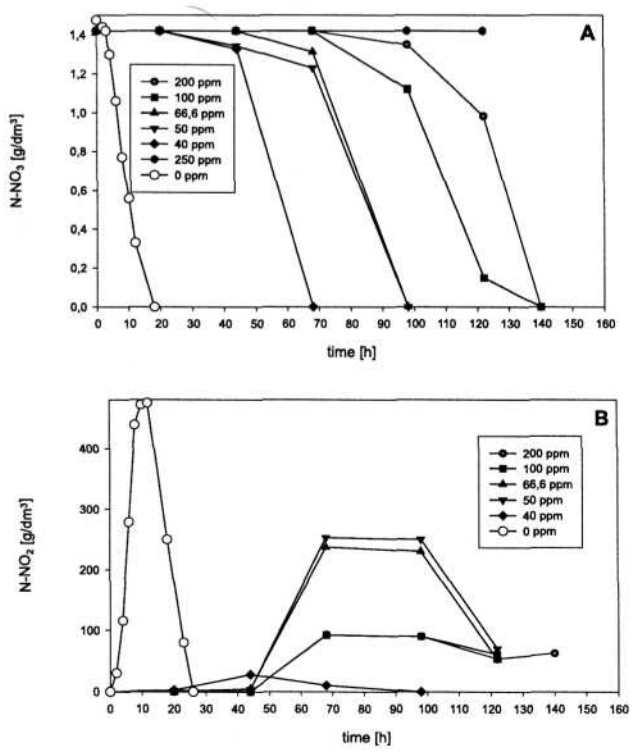


Fig. 3. The effect of l-bromo-3-chloro-5,5-dimethylhydantoine on denitrification process with *Bacillus licheniformis* bacteria A - reduction of nitrates to nitrogen, B - reduction of nitrites to nitrogen (37ºC; pH = 7.5; C/N = 2.33; BCDMH - solid substance).

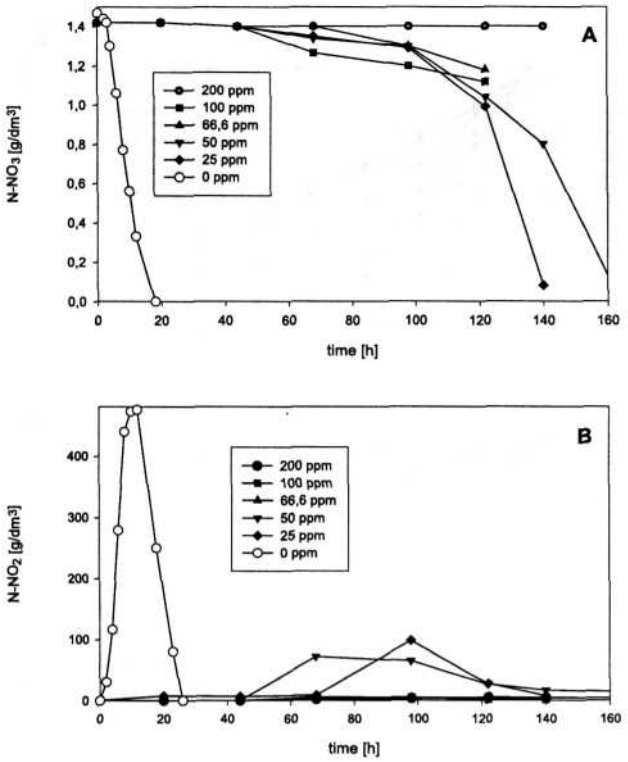


Fig. 4. The effect of concentration of l,3-dichloro-5,5-dimethylhydantoine on denitrification process with *Bacillus licheniformis* bacteria A - reduction of nitrates to nitrogen, B - reduction of nitites to nitrogen (37°C; pH = 7.5; C/N = 2.33; DCDMH -solid substance).

of denitrification is much lower, Fig. 4, and their decomposition occurs after about 140 hours.

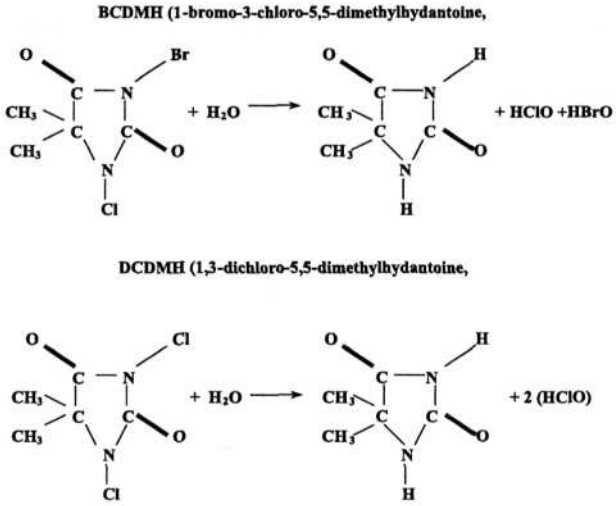
Microbiological activity of BCDMH and DCDMH against that of chloramine T given for the sake of comparison, are given in Table 2, which gives the range of tolerated concentrations, moderately toxic concentrations and toxic ones.

According to the data from Table 2, *Bacillus licheniformis* bacteria are more sensitive to the toxic influence of chloramine T than to that of BCDMH or DCDMH, although the calculated content of chlorine in chloramine T is lower than in DCDMH or BCDMH. This fact should be related first of all to a different rate of active chlorine release to the environment. In the case of DCDMH and BCDMH the rate of hydrolysis, so also the rate of halogen release, depends on the environmental conditions. The compounds are a source of concentrated active chlorine released in time as it is used up. Chloramine T is known to fast release chlorine when dissolved in water, therefore it is used for quick disinfection of water without the need for boiling [18].

The halogen derivatives of dimethylohydantoine are mild biocides which can compete with chlorine and such preparations as chlorinated lime [19]. Used as components of different preparations they are a safe and easy to use source of halogen introduced in a stable form [20, 21, 22].

The mechanism of the inhibiting activity, and later toxic effect on the micro-organisms involved in the processes of desulfurication and denitrification is related to the release of active halogen formed as a transient or final product of BCDMH or DCDMH decomposition. Hydrolysis of DCDMH and BCDMH leads to the appearance of chloric acid or chloric and bromic acid, respectively, so compounds of well recognised bactericidal properties [23].

The process of the halogen release in water environment can be put schematically as:



The biologically active HC1O or HBrO easier penetrate the cell membranes than ClO^- or BrO^- ions, and destroy the enzymatic system of bacteria. The ions ClO^- and BrO^- undergo hydrolysis in water and therefore their bactericidal ability is lower [23]. It has been established that the ClO^- ion is about 80 times less toxic than HClO

Table 2. Microbiological activity of BCDMH, DCDMH and chloramine T.

Bacteria	Compound	Concentration [ppm]		
		tolerated	inhibiting	toxic
Bacillus licheniformis	BCDMH	< 40	40 – 200	250
	DCDMH	< 25	25 – 100	200
	chloramine T	< 15	15 – 60	–
Desulfotomaculum ruminis	BCDMH	< 40	40 – 300	400
	DCDMH	< 40	40 – 200	300
	chloramine T	< 210	210 – 240	> 250

acid. The observed differences in bactericidal effectiveness of the compounds studied can be explained by a different rate of reaching a constant rate of hydrolysis depending on the progress of oxidation determined by environmental conditions. In the range of tolerated concentrations, see Table 2, the bacteria set on the protective and adapting mechanism, which helps them survive'. As follows from the study, the bacteria from the genus *Bacillus* are more sensitive to the biocides studied than *Desulfotomaculum ruminis.*

This is an interesting observation as in the study on the toxic effect of peroxides, e.g. magnesium monoperoxyftalate (MMPP) [24] the bacteria *Bacillus licheniformis* were more resistant than *Desulfotomaculum ruminis.* For the latter the lethal concentration of MMPP was 3.3 times lower than for *Bacillus licheniformis.* Therefore, *Desulfotomaculum ruminis* bacteria show lower abilities of adaptation to the environment with the reactive forms of oxygen but are more resistant to the presence of active chlorine, released from BCDMH or DCDMH, than *Bacillus licheniformis* bacteria. This observation is consistent with the results of earlier studies on the influence of trichloroisocyanuric acid (TCICA) and dichloroisocyanuric acid (DCICA) on proliferation of these bacteria [24],

In general, the tested compounds BCDMH and DCDMH can be treated as a safe source of slowly released halogen to water environment, and thus safe disinfectants [4-12]. Their use will reduce the amount of halogens released to the natural environment, which affect the natural processes of sulphur and nitrogen conversion taking place in ecosystems. Their use is thus not only economically justified but also beneficial for the natural environment.

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