# The Effect of TCICA on Denitrification and Desulfurication Processes

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## Abstract

The subject of this study is the effect of trichloroisocyanuric acid (TCICA) and sodium salt of dichloroisocyanuric acid (NaDCIC) - compounds used as disinfectants - on the kinetics of denitrification and desulfurication run with the use of bacteria from the *Bacillus* and *Desulfatomaculum* genera.

The inhibitory effect of the compounds tested on microbiological activity of the bacteria was evidenced and the limiting values of their admissible and toxic concentrations, which is of importance for protecting the natural environment against the influence of wastes formed after disinfection.

**Keywords;** Bacteria, *Bacillus licheniformis, Desulfatomaculum ruminis,* toxicity, denitrification, desulfurication, TCICA (trichloroisocyanuric acid), DCICA, (dichloroisocyanuric acid)

## Introduction

Trichloroisocyanuric acid (TCICA) and sodium salt of dichloroisocyanuric acid (NaDCIC) are used as convenient and effective means for oxidation and chlorination of organic compounds in experimental chemistry [1, 2, 3, 4] and as attractive disinfectants in households [5, 6, 7, 8].

These compounds disintegrate by producing an unstable chloric acid (I) HC1O, of known oxidizing and chlorinating properties [9, 10, 11]. A study on the kinetics of TCICA decomposition proves that the chloric acid (I) formed decomposes with the release of active oxygen or chlorine - depending on conditions [12, 13]. The range and efficiency of the bactericidal effect of TCICA depends on the concentration, time of contact, temperature and types of microorganisms in the environment [5-8].

According to literature, TCICA is most effective against the *Listeria* type bacteria carried by infected animals, food products and infected people, and in particular against factors producing bovine spongiform encephalopathy [6].

Common use of TCICA and its organic derivatives as household disinfectants prompted us to check its effect on the natural environment, to which it is transferred with household wastes. This would require determination of the degree of biodegradation of TCICA and products of its decomposition and the toxic concentration values which would help find methods of environmental protection against its effect on ecosystems.

This study was aimed at checking the influence of TCICA on the activity of bacteria taking part in the processes of denitrification [14] and desulfurication [15]. In particular, we studied the effect of TCICA, sodium salt of DCICA, and chloramine T (for the sake of comparison) on the kinetics of microbiological reduction of nitrates (V) and sulphates (VI) occurring with participation of bacteria from the genera *Bacillus* and *Desulfatomaculum.* Moreover, admissible and toxic concentrations of the tested compounds were determined.

## Materials and Methods

### Denitrification

The bacteria from the genus *Bacillus* taking part in the process of denitrification were isolated and identified as described in [16].

Kinetic study was performed at 37°C, pH 8, in sealed glass reactors of 20 $cm^3$ filled with 10 $cm^3$ of lactate medium of the following composition in [$g/dm^3$]: $N_{NO}3-$ = 1-40, $Fe(NO_3)_3$ x $9H_2O$ = 0.44, $NH_4Cl$ = 0.25, $MgSO_4$ x 7 $H_2O$ = 0.50, $CaCl_2$ = 1.00, $Na_2HPO_4$ x $12H_2O$ = 2.50, $C_{org.}$ =s 2.52 and microelements. 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: TCICA or NaDCIC, in different concentrations up to 10 ppm, was added. The influence of chloramine T used in concentrations from 10 to 15 ppm was also studied. Denitrification rate was determined by periodical measurements of the concentration of nitrates (V) and nitrites (III).

### Desulfurication

The bacteria-reducing sulphates (VI) were isolated and identified as *Desulfotomaculum ruminis* by the method described in [17].

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 [$g/dm^3$]: $MgSO_4$ x $7H_2O$ = 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. The medium was inoculated with 4%v of the inoculum collected after 24 hours of the bacteria growth (logarithmic growth phase). The compounds tested (TCICA and NaDCIC) were added in different concentrations up to 92 and 130 ppm, respectively, and for the sake of comparison the effect of chloramine T was tested in concentrations up to 250 ppm. The rate of the reaction was determined by the periodically measured amount of sulphites to which sulphates (VI) were reduced.

The instruments and media used in the experiment were sterilized for 20 min at 120ºC. In the same conditions, parallel experiments were carried out on the reference samples (without the compounds tested). The data given in Table 1 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.

Table 1. Microbiological activity of trichloroisocyanuric acid (TCICA), sodium salt of dichloroisocyanuric acid (NaDCIC) and chloramine T.

| Bacteria | Compound | Concentration [ppm] | | |
|---|---|---|---|---|
| | | tolerated | inhibiting | toxic |
| *Bacillus licheniformis* | TCICA | < 2 | 2-8 | > 10 |
| | NaDCIC | < 2 | 2-8 | > 10 |
| | chloramine T | < 15 | 15-60 | - |
| *Desulfotomaculum ruminis* | TCICA | < 75 | 75-90 | > 92 |
| | NaDCIC | < 110 | 110-128 | > 130 |
| | chloramine T | < 210 | 210-240 | > 250 |

### Methods of Analysis

The concentration of nitrates (V) were measured by the potentiometric method using an ion-selective electrode "Detector".

The concentration of nitrates (III) were determined spectrophotometrically on a Beckman DU-640 spectrometer at λ = 520nm [18].

The concentration of sulphides was found in the precipitated CdS by the iodometric method [19].

The concentration of sulphates (VI) was determined by the complexometric method [19].

## Results and Discussion

The bactericidal activity of TCICA, NaDCIC and chloramine T (substances used as disinfectants) is related to the formation of the so-called useful chlorine in the form of HClO, $ClO^-$ or $Cl_2$ in water environments. The efficiency of the above preparations depends on their concentration, environmental conditions and the kind of microorganisms against which they are used. The influence of TCICA on the kinetics of desulfurication is illustrated in Fig. 1.

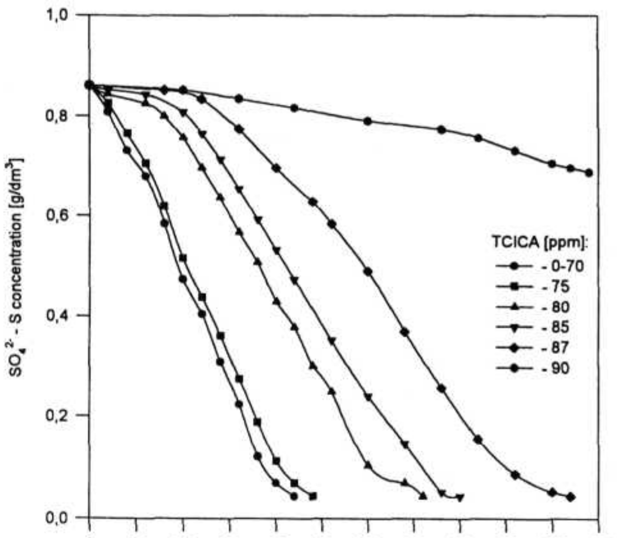



Fig. 1. The effect of TCICA concentration on microbiological reduction of sulfates with *Desulfotomaculum ruminis* bacteria (T = 37ºC, C/S = 9.3, pH = 6.8 - 7.2).

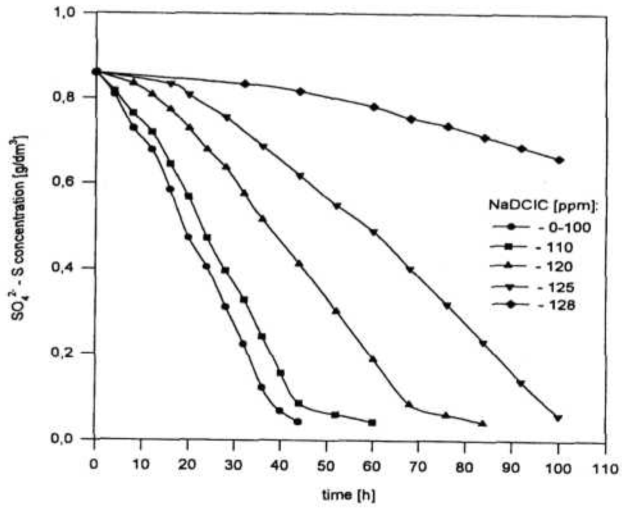



Fig. 2. The effect of NaDCIC concentration on microbiological reduction of sulfates with *Desulfotomaculum ruminis* bacteria (T = 37ºC, C/S = 9.3, pH = 6.8 - 7.2).

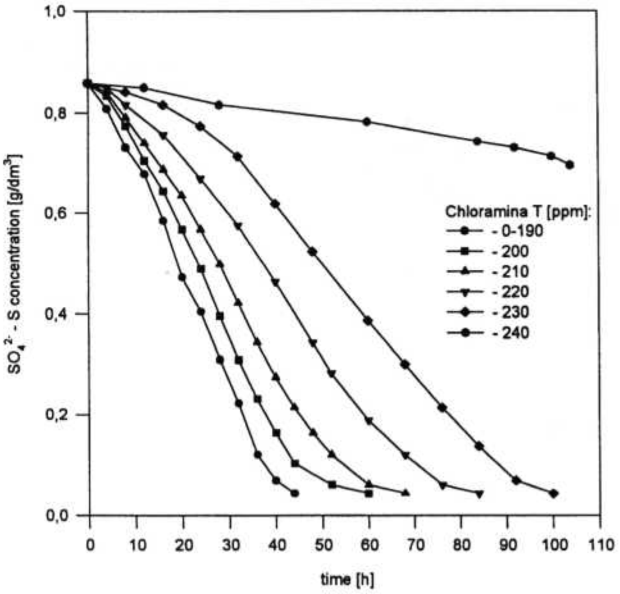



Fig. 3. The effect of chloramina T concentration on microbiological reduction of sulfates with *Desulfotomaculum ruminis* bacteria (T = 37ºC, C/S = 9.3, pH = 6.8 - 7.2).

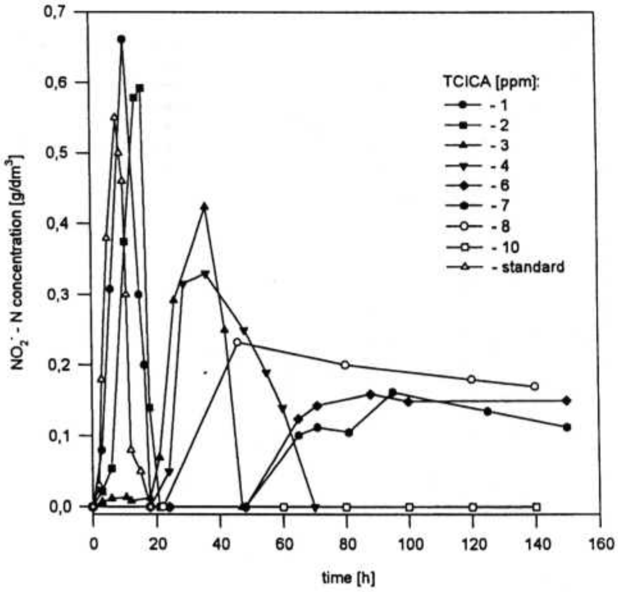



Fig. 5. The effect of TCICA concentration on microbiological reduction of nitrites with *Bacillus licheniformis* bacteria (T = 37ºC, C/N = 2.1, pH = 7.5 -8.0).

According to the data presented in Fig. 1, up to a concentration of 75 ppm TCICA does not change the activity of *Desulfotomaculum ruminis* bacteria. Increasing its concentration to 87 ppm systematically prolongs the process of desulfurication, which is evidence of its inhibiting influence. At the concentration of 90 ppm, TCICA causes a significant inhibition of the process and above this concentration bacteria die and the process is irreversibly terminated.

The influence of NaDCIC is similar as only the range of the inhibiting effect is shifted towards higher concentrations.

The bactericidal effect resulting in irreversible inhibition of desulfurication is observed at the concentrations above 128 ppm NaDCIC.

The effect of chloramine T, tested for the sake of comparison, proved insignificant when used at concentrations of 190 ppm, and only above 240 ppm was found to be toxic for *Desulfotomaculum* bacteria.

The *Bacillus licheniformis* bacteria used in the denitrification process are more sensitive to the toxic effect of the compounds tested than *Desulfotomaculum ruminis.* Already at the concentration of 1 ppm TCICA causes a noticeable decrease in reduction of nitrates (V), Fig. 4. In the range of 2 to 8 ppm the inhibiting effect is noticeable and at 10 ppm TCICA is toxic for the bacteria studied.

Fig. 5. illustrates the influence of TCICA on the kinetics of reduction of nitrates (III), which are the intermediate products of denitrification.

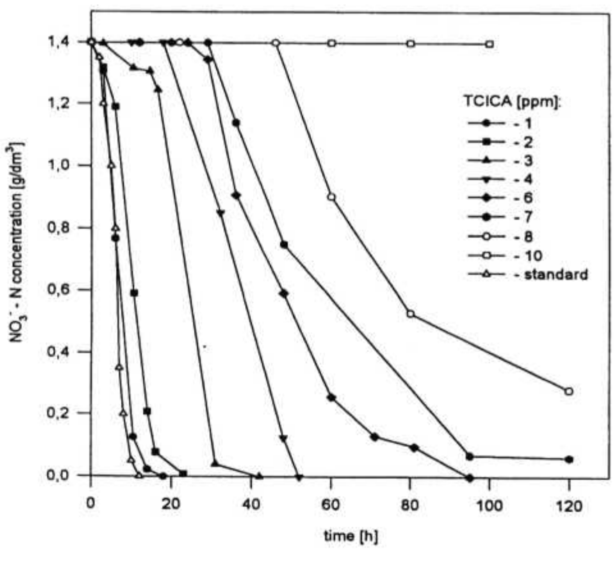



Fig. 4. The effect of TCICA concentration on microbiological reduction of nitrates with *Bacillus licheniformis* bacteria (T = 37ºC, C/N = 2.1, pH = 7.5 - 8.0).

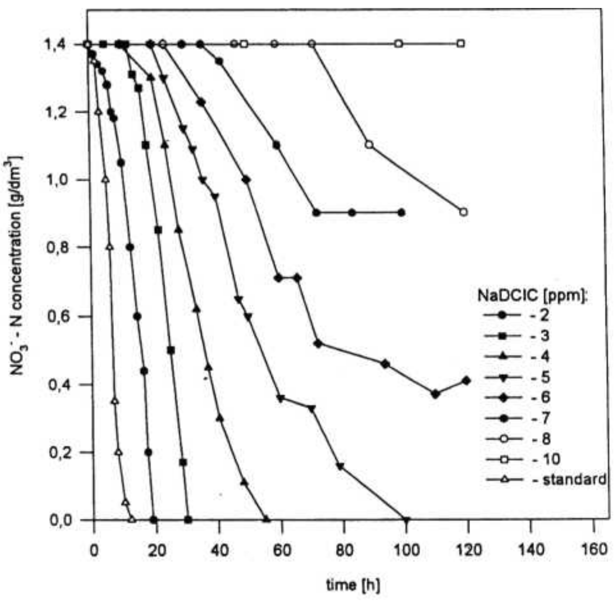



Fig. 6. The effect of NaDCIC concentration on microbiological reduction of nitrates with *Bacillus licheniformis* bacteria (T = 37ºC, C/N = 2.1, pH = 7.5 - 8.0).

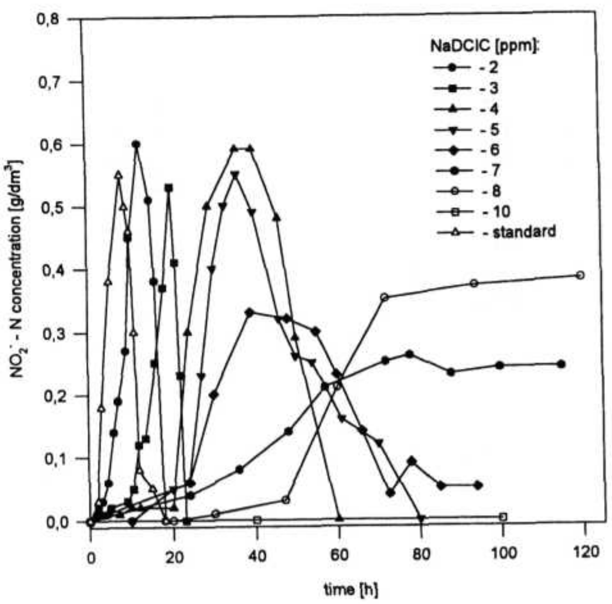



Fig. 7. The effect of NaDCIC concentration on microbiological reduction of nitrites with *Bacillus licheniformis* bacteria (T = 37°C, C/N = 2.1, pH = 7.5 - 8.0).

According to the results shown in Fig. 5, TCICA at the concentration of 6-8 ppm causes inhibition of denitrification at the stage of toxic nitrates (III), which remain undecomposed in the medium at concentrations below 0.2 $g/dm^3$

NaDCIC shows a similar effect on the *Bacillus licheniformis* bacteria. When introduced at the concentration of 10 ppm this causes irreversible termination of the process of reduction of nitrates (V).

The reduction of nitrates (III) to free nitrogen in the medium containing 7 and 8 ppm NaDCIC is inhibited, respectively, at the level of 0.23 and O.38g/$dm^3$.

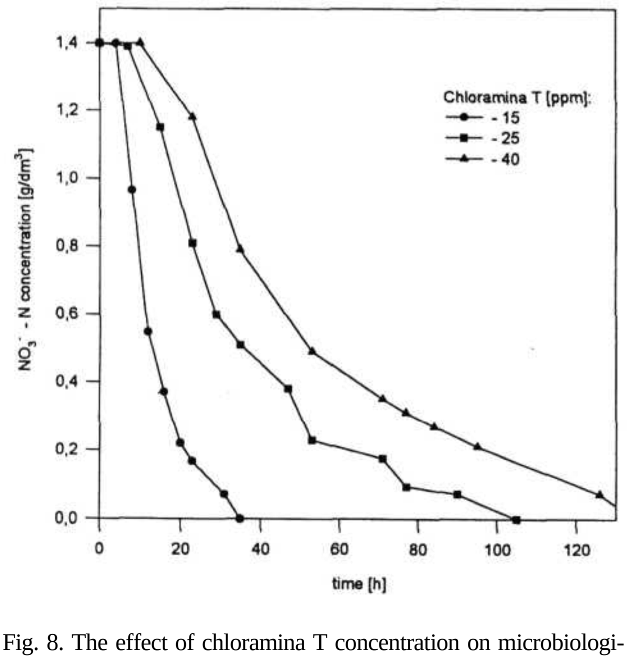



Fig. 8. The effect of chloramina T concentration on microbiological reduction of nitrates with *Bacillus licheniformis* bacteria (T = 37ºC, C/N = 2.1, pH = 7.5 - 8.0).

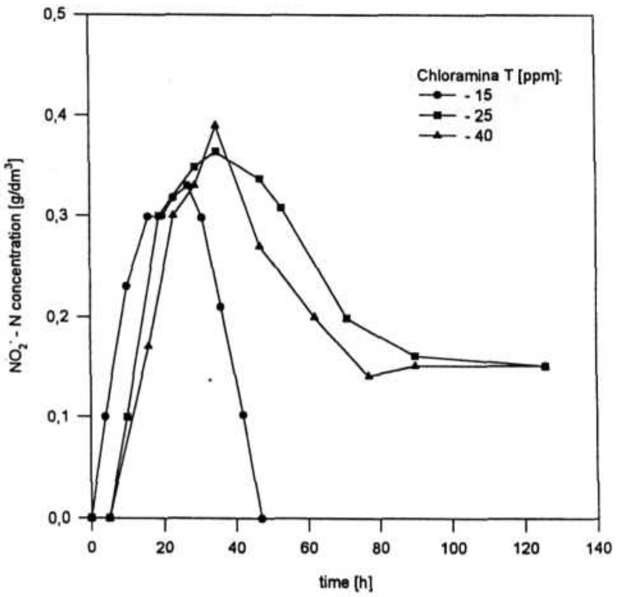



Fig. 9. The effect of chloramina T concentration on microbiological reduction of nitrites with *Bacillus licheniformis* bacteria (T = 37°C, C/N = 2.1, pH = 7.5 - 8.0).

Chloramine T proves highly toxic for *Bacillus licheniformis* bacteria, already at 15 ppm it prolongs the time of denitrification from 18 h (for the reference sample) to 38 h.

The presence of chloramine T at concentrations of 25 and 40 ppm, reduction of $NO_2^-$ to free nitrogen is inhibited at the level of about 0.15 $mg/dm^3$.

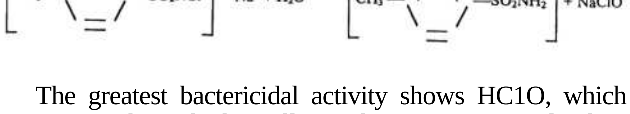
As follows from the above results, TCICA has the highest bactericidal ability.

The mechanism of the inhibiting and toxic effect of the compounds studied on the microorganisms used in the processes of desulfurication and denitrification undoubtedly involves formation of the so-called useful chlorine, which is the final or intermediate product of their decomposition **[11].** Apart from isocyanuric acid $(HNCO)_3$ and chloric acid (I) of known bactericidal properties, is formed [20]. According to the results reported in [12, **13],** the processes of TCICA and NaDCIC hydrolysis occur in the following reactions:

$$\mathrm{TCICA + H_2O \underset{}{\overset{k_1}{\rightleftharpoons}} DCICA + HClO}$$

$$\mathrm{DCICA + H_2O \underset{}{\overset{k_2}{\rightleftharpoons}} MCICA + HClO}$$

Monochloroisocyanuric acid can be further decomposed to the final isocyanuric acid. Similarly, decomposition of chloramine T leads to the formation of HClO:




The greatest bactericidal activity shows HC1O, which penetrates through the cell membranes more easily than $ClO^-$, and attacks the enzymatic system of the bacteria. The $ClO^-$ ions are hydrated and their bactericidal ability is weaker [20]. The differences in bactericidal efficiency between the compounds studied are related to a different rate constant of hydrolysis processes [13]. As follows from the kinetic studies [13], the rate constant of TCICA decomposition (k,) is about twice higher than that of NaDCIC decomposition ($k_2$), which means that in the former reaction the concentration of HClO formed is higher, and thus the toxic effect of TCICA is stronger. Moreover, as follows from [20], $ClO^-$ ions are about 80 times less toxic than weak chloric acid (I) HClO. The prolongation of the inductive period observed with increasing concentration of the compounds tested is a consequence of prolonged processes of the bacteria adaptation to environmental conditions.

The ranges of tolerated, inhibiting and toxic concentrations of TCICA, NaDCIC and chloramine T are given in the Table 1.

In the range of tolerated concentrations the defence and adaptive mechanisms effectively protect the bacteria against destruction.

In the range of inhibiting concentrations the influence of the products of hydrolysis of the compounds studied becomes more effective and finally toxic.

The most sensitive to these compounds are the *Bacillus licheniformis* bacteria, for which the toxic effect of TCICA and NaDCIC was observed at concentrations of the latter of 10 ppm. It may be of interest to remind you that as we showed in [21], *Bacillus licheniformis* were the least sensitive to the toxic effect of peroxides, e.g. magnesium monoperoxyphtalate (MMPP), while the *Desulfotomaculum ruminis* bacteria showed much weaker adaptive abilities to the environment with reactive forms of oxygen. The lethal concentration of MMPP was for *Desulfotomaculum ruminis* 3.3 times lower than that for *Bacillus licheniformis.*

The compounds tested: TCICA, NaDCIC and chloramine T used as disinfectants were proved to reduce the activity of the microorganism studied already at low concentrations. Their effect is particularly strong for the *Bacillus licheniformis* bacteria. The above mentioned compounds when present in the environment in concentrations higher than the toxic values can threat to inhibit the natural cycle of sulphur and nitrogen. Prevention of contamination of the natural environment by these compounds is possible by controlling their concentrations in waste.

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