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INFLUENCE OF PRELIMINARY OXIDATION OF MODEL WATER WITH CHLORINE DIOXIDE ON ITS MUTAGENICITY

Chlorine-disinfected model water after preliminary oxidation with chlorine dioxide of different composition was used in investigations. Extracts from model water samples with different TOC and NH_4^+ ions after elution with acetone were applied in the *Salmonella* assay in TA100 test strain. The higher the concentration of those parameters, the higher the concentration of such chlorine derivatives as AOX and chlor(o)acetic acids in water, and the stronger the mutagenic activity of organic water pollutants observed in the tests conducted without metabolic activation. Only water that contained less TOC than 2.0 g/dm^3, irrespective of the ammonia concentration (0.1; 0.5; 1.0 g NH_4^+/dm^3), did not show mutagenic effects in tests conducted either with or without metabolic activation.

1. INTRODUCTION

The concentration of chemical and bacterial pollutants is gradually growing in the surface and infiltration waters which are used for water supply purposes in bigger and bigger quantities than underground waters. The insufficient removal of these pollutants, which are definitely harmful for the health of the consumers, in the water purification processes requires the use of large quantities of disinfectants, as the ultimate objective of the water treatment process is to provide water without microbiological pollutants [1], [2].

The disinfection of water containing natural and antropogenic organic compounds with chemical oxidants generates the production of numerous, not fully known by-products which are characterized by biological activity, including carcinogenicity. Most of them develop as a result of chlorine-disinfection [3], [4]. That is why other disinfection methods, such as, e.g., preliminary application of chlorine dioxide, are so frequently introduced before proper chlorination.

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2. RESEARCH METHODS

The research was conducted under laboratory conditions in chlorine-disinfected model water of different composition after its preliminary oxidation with chlorine dioxide in the amount of 0.4 mg ClO_2/dm^3 according to the chlorine demand curve for water.

The model water was prepared from tap water after its purification with active carbon and from the water rich in natural dissolved humus compounds from Batorow Great Peats. The proportion of these waters are such that TOC is equal to 2.0, 4.0, 6.0 mg C/dm^3. The solution of ammonium chloride was added to the waters of a given TOC to obtain the following concentration of NH_4^+: 0.1, 0.5, 1.0 mg NH_4^+/dm^3. The tap water used in the tests was the surface water taken from the Oława River and purified in Water Treatment Plant "Mokry Dwór" in Wrocław in the processes of coagulation and filtration on sand deposits and subsequently disinfected.

Based on the earlier findings reported by TRACZEWSKA et al. [5], the water samples were concentrated in the parallel system with the use of Amberlite XAD resins of different adsorption capabilities in the sequence determined experimentally: XAD 16, 7 and 2. A 10-dm^3 water sample was filtered through the set of columns and organic compounds adsorbed on resins were extracted with acetone. The extracts were then subjected to bioindicative tests.

The mutagenic effect of the organic fraction extracted from model water was assessed carrying out the Ames test [6] on *Salmonella typhimurium* TA100 test strain. This strain detects the presence of mutagens that cause base-pair substitution. The number of spontaneous revertants of *S. typhimurium* TA100 fell between 100 and 150 per plate. The tests were conducted with and without metabolic activation of promutagens with the use of S9 microsomal fraction from the rat liver activated with Aroclor 1254. The compounds present in the water samples were used in the *Salmonella* assay. The water samples were concentrated in such a way as to reach the following concentration rates: 1000, 800, 600, 500, 400, 300, 200 and 100, which corresponded to the water volume ranging from 10 to 100 cm^3. Each test was conducted in five replications.

Based on the results obtained in *Salmonella* assay, the mutagenicity rate (MR) was assessed. Any induced reversion being at least twice as high as a spontaneous one, i.e. when MR was greater or equal to 2, was considered to be positive for a given water sample.

After its concentration, each model water was tested for the presence of chloroorganic compounds (AOX) and chlor(o)acetic acids (HAA), including mono-, di- and tri-chlor(o)acetic acids. The analytical methods included tests for: total organic carbon (TOC) with the use of Shimadzu TOC 5050; chlorine, with the use of the iodometric method [7]; chlorinated organic compounds as AOX with the Nanocolor Test 0-07, Macherey-Nagel; chlor(o)acetic acids (HAA), the USEPA 522.2 procedure with the use of Shimadzu 17 A gas chromatograph.

3. RESULTS

The research examined the dependence of the content of TOC in model water, concentration of NH_4^+ as well as the doses of chlorine dioxide and chlorine on the secondary water pollutants that develop after disinfection (AOX, chlor(o)acetic acids: MClAA, DClAA, TClAA). Biological activity of these secondary pollutants was assessed as well.

A normal dose of chlorine applied in the tests increased along with the increase in the content of TOC and NH_4^+ concentration from 2.25 to 14.0 mg Cl_2/dm^3 (figure 1). The proportion of D_1 in its normal dose was from 44 to 90% and grew along with the increase in TOC and NH_4^+, whereas the proportion of D_2 ranged from 10 to 56% and did not demonstrate such a relation.

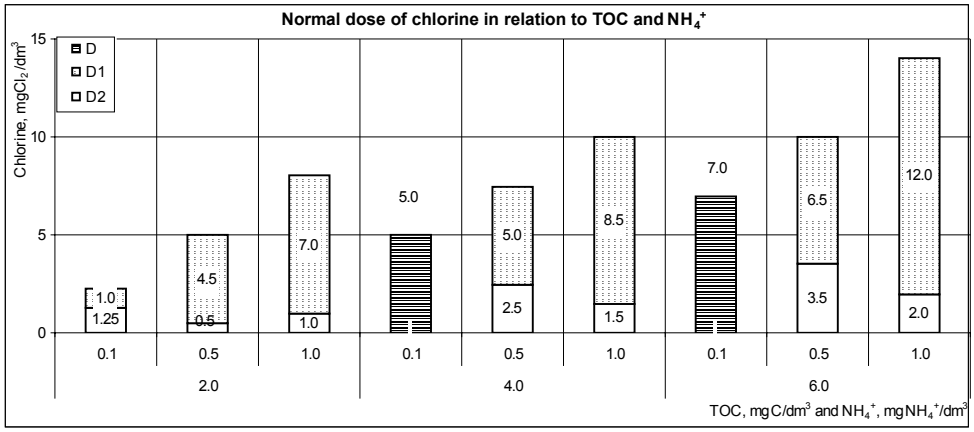

Fig. 1. Normal dose of chlorine applied in chlorination of model water after its preliminary oxidation with chlorine dioxide

The disinfection of model water was responsible for the formation of chlorine derivatives of organic compounds. An increase in the concentration of AOX from 47 μg Cl/m^3 to 244 μg Cl/m^3 was detected in model water after its concentration (figure 2). On the other hand, the sum of chlor(o)acetic acids in model water after its concentration ranged from 23.8 μg Cl/m^3 (at the lowest content of TOC and the lowest concentration of NH_4^+) to 91.6 μgCl/m^3 (with the highest content of TOC and the highest concentration of NH_4^+). The concentration of mono-, di- and tri-chlor(o)acetic acids in model water increased along with the increase in the content of TOC and the concentration of NH_4^+ from 5.6 μg Cl/m^3 (TClAA) to 36.8 μg Cl/m^3 (MClAA) (figure 3). After concentration of model water the proportion of the sum of chlor(o)acetic acids in absorbable halogenated organic compounds was on average 39% (35–51%).

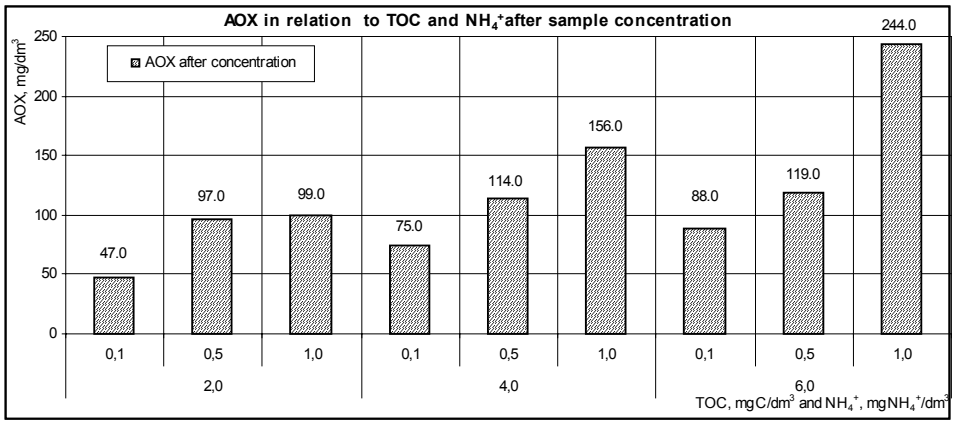

Fig. 2. Absorbable halogenated organic compounds developed after disinfection of model water with chlorine and preliminary oxidation with chlorine dioxide

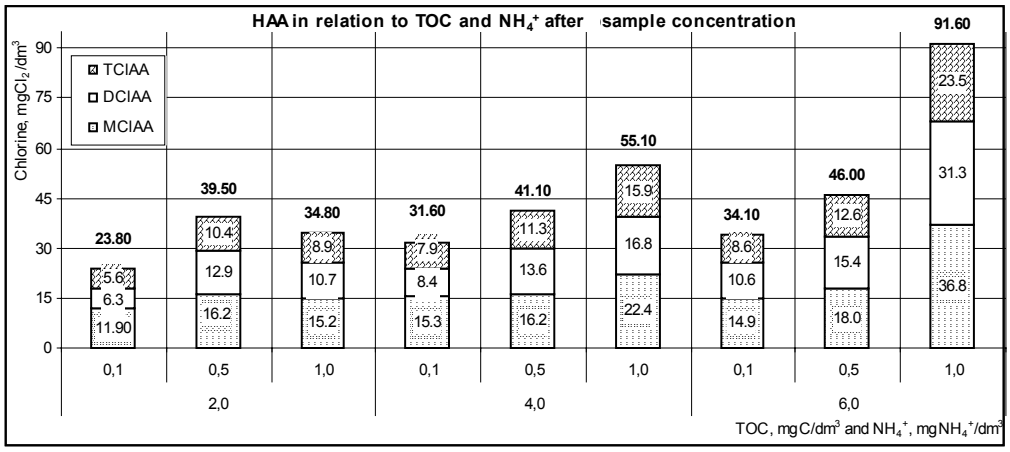

Fig. 3. Sum of chlor(o)acetic acids (mono-, di-, tri-chlor(o)acetic) developed after disinfection of model water with chlorine and preliminary oxidation with chlorine dioxide

The extracts of chlorine-disinfected model water of different composition after its preliminary oxidation with chlorine dioxide were tested using the *Salmonella* assay, which allowed us to determine the concentrations of TOC and NH_4^+ at which potentially mutagenic pollutants can be expected in drinking water.

The tests with and without metabolic activation conducted on the extracts from model water with a 2.0 mg/dm^3 content of organic carbon for three concentrations of NH_4^+, i.e. 0.1, 0.5 and 1.0 mg NH_4^+/dm^3, did not demonstrate MR above 2.0 (figure 4b). MR at the level close to unity recorded in those tests proves that at such parameters of

water no by-products of disinfection develop which could affect the genetic material of bacterial cells.

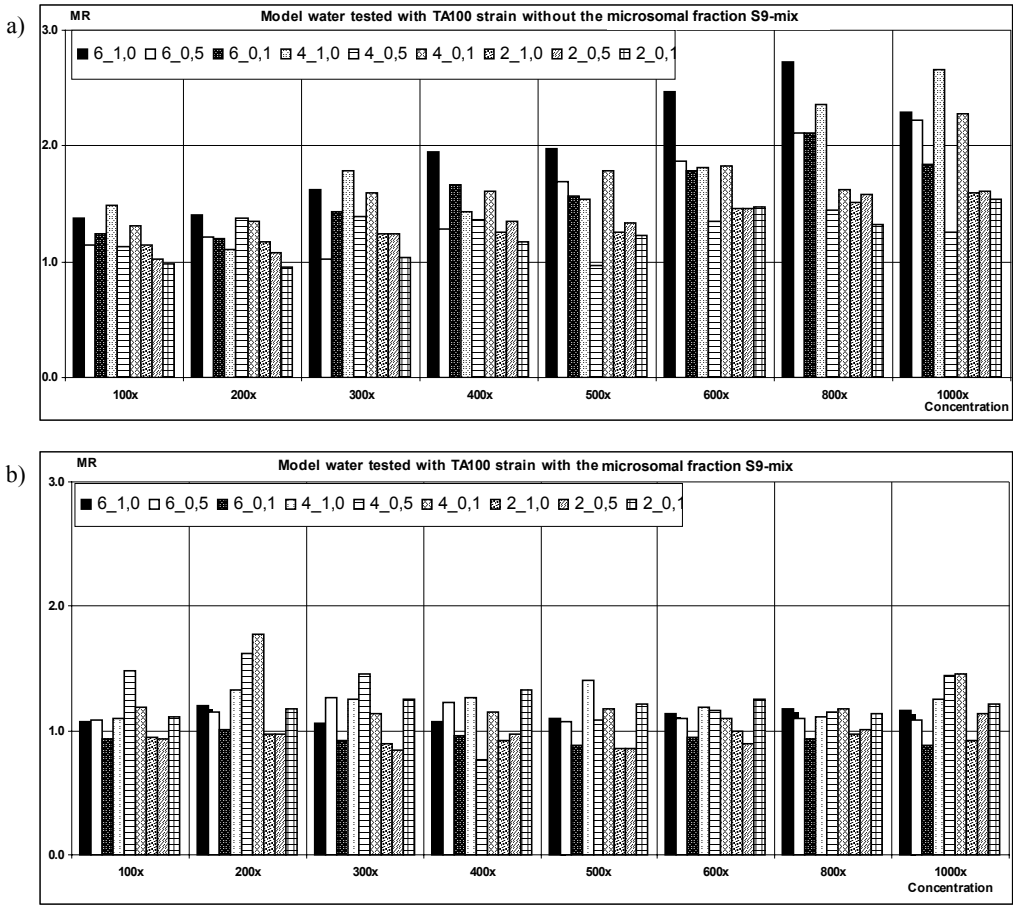

Fig. 4. MR of micropollutants in model water disinfected with chlorine at TOC of 6.0, 4.0 and 2.0 mg C/dm^3 and three different concentrations of NH_4^+: 0.1, 0.5 and 1.0 mg NH_4^+/dm^3

In the case of model water containing TOC from 4.0 to 6.0 mg C/dm^3, irrespective of the ammonia concentration (ranging from 0.1 to 1.0 mg NH_4^+/dm^3), the extracts tested demonstrated potential mutagenic effect in the tests conducted without metabolic activation (figure 4a).

MR increased with an increase in TOC content and NH_4^+ concentration. Only the results obtained for model water at TOC = 4.0 mg C/dm^3 and 0.5 mg NH_4^+/dm^3 slightly deviate from the general trend observed during the tests.

In the extract from chlorinated model water with TOC of 4.0 mg C/dm^3 and 0.1 mg NH_4^+/dm^3, the value of MR was above 2 at the concentration rate of 1000. At the same concentration of organic compounds and 1.0 mg NH_4^+/dm^3, the Ames test had positive results for the water whose concentration rose 800× and 1000×.

In the case of chlorine-disinfected and preliminary oxidated model water with TOC of 6.0 mg C/dm and 0.1 mg NH_4^+/dm^3, the Ames test gave positive results only if water concentration rose 800×. In the test with the extracts from water of the highest content of TOC (6.0 mg C/dm^3) and 0.5 mg NH_4^+/dm^3, the micropollutants in the water concentrated 800× and 1000× caused the mutagenic effects in the tests conducted without S9 fraction. At TOC of 6.0 mg C/dm^3 and 1.0 mg NH_4^+/dm^3, the test had positive results at the water concentrated from 400× to 1000×.

The extracts from model water at TOC of 4.0 and 6.0 mg C/dm^3 did not produce mutagenic effect in the tests conducted with metabolic activation. Probably the microsomal enzymes in S9-mix fraction changed the structure of the compounds, hence they were not mutagenic.

4. SUMMARY

Surface waters necessitate an effective treatment, e.g. by chlorination, which is responsible for the formation of noxious disinfection by-products, potentially mutagenic and carcinogenic, despite the preliminary oxidation of model water with chlorine dioxide.

The tests conducted demonstrated that normal doses of chlorine increased with an increase in TOC content and NH_4^+ concentration. These increased doses of chlorine caused in turn an increase in AOX concentration. The concentration of individual chlor(o)acetic acids (mono-, di- and tri-chlor(o)acetic acids) also increased with an increase in TOC content and NH_4^+ concentration.

The *Salmonella* test conducted with the use of TA100 strain with and without microsomal fraction on the extracts from chlorine-disinfected model water samples of different content of TOC and NH_4^+, after their elution with acetone, allowed us to assess the potential mutagenic effects of water micropollutants as both direct and indirect mutagens that cause a base-pair substitution. The mutagenicity rate (MR) for the test extracts from water increased with an increase in TOC content and NH_4^+ concentration. For model water with TOC of 4.0 and 6.0 mg C/dm^3, the positive results of the tests were obtained only in tests conducted without metabolic activation (the presence of direct mutagens). The highest values of MR were observed in the water with the highest concentration of test parameters (TOC = 6.0 mg C/dm^3, NH_4^+ = 1.0 mg NH_4^+/dm^3). The *Salmonella* tests conducted with metabolic activation on these extracts had negative results, which testified to the absence of promutagenic compounds in test waters.

On the other hand, model water with organic carbon content of 2.0 g C/m^3, regardless of NH_4^+ concentration, did not show any potential mutagenic effects.

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WPŁYW WSTĘPNEGO UTLENIANIA WODY MODELOWEJ DWUTLENKIEM CHLORU NA JEJ MUTAGENNOŚĆ

Wodę modelową o różnym składzie wstępnie utleniano dwutlenkiem chloru, a następnie dezynfekowano chlorem. Do testu *Salmonella* przeprowadzanego na szczepie TA100 wprowadzano ekstrakty z próbek wodnych. Ekstrakty te wymywano acetonem z wód modelowych o różnej zawartości OWO i jonów amonowych. Wraz ze zwiększeniem się stężenia OWO i jonów amonowych wzrastało w wodzie stężenie chloropochodnych takich jak AOX i kwasy chlorooctowe oraz mutagenne działanie organicznych zanieczyszczeń wody w testach przeprowadzanych bez aktywacji metabolicznej. Jedynie woda o zawartości OWO 2,0 g C/dm^3, niezależnie od stężenia NH_4^+ (0,1; 0,5; 1,0 g NH_4^+/dm^3), zarówno w testach z aktywacją metaboliczną, jak i bez niej nie wykazywała właściwości mutagennych.