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Granular polymers with immobilized *N*-chlorosulfonamide groups as alternative water disinfectants

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ABSTRACT

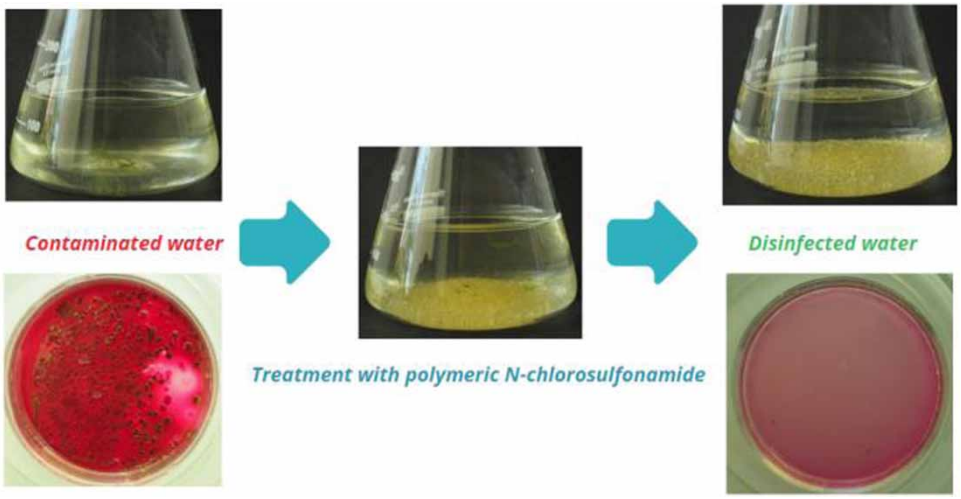
In light of the deterioration of microbiological composition of natural and technical water, the development of new approaches to its disinfection is an important technological task. The use of chlorine-active compounds remains the most effective for this purpose, but traditional preparations such as sodium hypochlorite pose a number of environmental risks. This paper describes the processes of treating model microbiologically contaminated solutions with granular styrene-divinylbenzene polymers with immobilized *N*-chlorosulfonamide groups. In this case, chlorine is released from the polymer surface into the solution due to chlorination of the amine components of the microbial cell. The amount of chlorine released is proportional to the degree of microbial contamination. The main factors influencing the disinfection rate and the characteristics of the chlorine emission process are the intensity of stirring, the type and concentration of the microorganism, and the surface area of the polymer. The treatment is effective against individual Gram-positive and Gram-negative bacteria, including multi-resistant ones, fungi, and multi-culture natural media. The use of this method for water disinfection potentially allows avoiding chlorine overdose, minimizing the formation of toxic chlorine-containing by-products, and ensuring long-term protection of water from re-contamination during storage.

Key words: active chlorine, antimicrobial polymers, chlorine release, *N*-chlorosulfonamides, sodium hypochlorite, water disinfection

HIGHLIGHTS

- Polymers with immobilized *N*-chlorosulfonamide groups can suppress microorganisms suspended in water.
- The amount of chlorine released from the polymer is proportional to the degree of microbial contamination.
- The key parameters of the process depend on stirring, interphase surface, and microbiological composition.
- Immobilized functional groups demonstrate high stability during long-term exposure to contaminated model solutions.

GRAPHICAL ABSTRACT



INTRODUCTION

According to the World Health Organization (WHO), 25% of the global population did not have access to safe drinking water in 2020, and this situation tends to worsen (Wang *et al.* 2024). Therefore, the development and implementation of new effective water treatment technologies is one of the most important scientific and applied tasks. Impurities of not only chemical, but also microbiological origin (Kristanti *et al.* 2022) pose a danger. Water from both natural and artificial sources is a favorable environment for the development of various microorganisms, including highly pathogenic ones (Hallsworth 2021). Thus, waterborne diarrheal diseases are one of the most common causes of morbidity and mortality around the world, especially in developing countries, taking, according to various estimates, from 800 to 1,800 thousand lives per year and causing significant economic losses (Meki *et al.* 2022). Well-known and dangerous (especially for children) diseases transmitted through water include cholera, campylobacteriosis, salmonellosis, amebiasis, as well as a number of diseases caused by adeno-, astro-, entero-, and other viruses (Arnone & Perdek Walling 2006; Woodall 2009). The predicted global temperature increase of 2 °C by 2040 will certainly only contribute to the growth of microbial contamination of water (Dupke *et al.* 2023). In addition, the development of superbacteria in water and the active horizontal transfer of antibiotic-resistance genes between waterborne microorganisms are threatening factors (Baquero *et al.* 2008; Odonkor & Addo 2018). Thus, the widespread use of disinfection technologies is vital to prevent serious consequences associated with deterioration in water quality.

Water disinfection can be carried out using various methods, both physical and chemical (Lanrewaju *et al.* 2022; Grzegorzek *et al.* 2023). A common method is membrane filtration, the most effective type of which is reverse osmosis (Razali *et al.* 2023). The disadvantage of this approach is the need to create high water pressure, regular cleaning/replacement of membranes, and careful preliminary preparation of water to avoid rapid pore clogging. Another popular physical method of water disinfection is UV irradiation, but this technology requires a powerful radiation source and is ineffective against certain viruses (Kim *et al.* 2022). Promising, but less common physical methods are cavitation (Yadav *et al.* 2021) and photocatalytic (Zhang *et al.* 2023) disinfection.

However, the most effective, technologically simple, and widely used method is chemical disinfection of water, in which chlorine compounds play a key role. Traditional preparations include molecular chlorine, sodium hypochlorite (NaOCl), various *N*-chloramines, and recently chlorine dioxide (Haida Nadia Mohamed Jefri *et al.* 2022; Lindmark *et al.* 2022; Nielsen *et al.* 2022). Due to their high oxidizing and chlorinating capacity, these compounds have a wide spectrum and high rate of microbiocidal action, and the development of microbial resistance to them is not typical. In addition, they can be produced in industrial quantities relatively simply and cheaply from available raw materials. Such preparations are used both in high-power industrial installations and for express water purification in domestic conditions (for example, tablets with *N*, *N*-dichloroisocyanurate (Jain *et al.* 2010)). However, a serious drawback of using chlorine-based agents is the formation of toxic by-products, especially when they are used in high concentrations in water with a high content of organic impurities (Srivastav *et al.* 2020). Effective antimicrobial concentrations of chlorine-active compounds and their acceptable residual

levels in drinking water are known and amount to up to 5 mg/L in different countries (García-Ávila *et al.* 2021; Onyutha & Kwio-Tamale 2022). During industrial water treatment, a corresponding amount of disinfectant (most often NaOCl) is usually added without preliminary microbiological analysis of the water. In most cases, the preparation is used in excess (sometimes significant) to compensate for the decomposition of free chlorine during a reaction with organic impurities (Kwio-Tamale & Onyutha 2024). At the same time, it is obvious that the amount of disinfectant can be reduced when treating water with a low organic and microbial load, which will lead to a decrease in undesirable side effects of the purification process and resource savings. When using organic *N*-chloramines, reducing the amount of the preparation administered into the water will reduce the likelihood of allergic effects and minimize deviations in consumer qualities, for example, in taste. On the other hand, insufficient active chlorine concentration may not allow achieving complete disinfection. Thus, determining and maintaining the correct concentration of chlorine disinfectant in water, ensuring an optimal balance between a satisfactory microbiocidal effect and the risk of unacceptable side processes, is a complex technological task.

Previously, we developed methods for the synthesis and studied the microbiocidal properties of polymers with immobilized *N*-chlorosulfonamide groups, and also showed that they can release active chlorine into water containing amine activators (Murashevych *et al.* 2020, 2021, 2023, 2024a, b). Styrene-divinylbenzene granules, originally intended for the production of cation exchangers for water treatment and therefore possessing the appropriate physical and mechanical characteristics, are used as a carrier polymer for such materials. The most important feature of the synthesized polymers is their ability to release the active agent into the environment in an amount equivalent to the amount of activator, which can theoretically solve the problem of active chlorine overdose in the treated water. In this work, we consider the processes of disinfection of model solutions using such polymers under various conditions with reference to the accumulation of chlorine compounds in the water sample.

METHODS

Polymer materials used and their analysis

Granular styrene-divinylbenzene polymers with immobilized *N*-chlorosulfonamide groups in the Na-form were used in the work. The polymers were synthesized from commercially available gel cation exchanger Purolite C100 using the method described in Murashevych *et al.* (2024a). Two samples of the same chemical nature were studied, but with different contents of immobilized active chlorine – 5.2 and 9.4%. The physicochemical properties and spectral characteristics of the polymers coincided with those described earlier. The main parameters of the materials used are given in Table 1.

The content of active chlorine immobilized on the polymer was determined before and after each experiment using a specially developed method, which includes its activation with taurine (Murashevych *et al.* 2023).

Microorganisms used in the study

The study used reference strains *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Candida krusei* ATCC 628, obtained from the museum of living cultures of the Dnipro State Medical University, as well as multi-resistant clinical isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, described in our article (Stepanskyi *et al.* 2024).

Table 1 | Main properties of the polymers used

Parameter	Value
Polymer carrier	Copolymer of styrene with divinylbenzene in gel granular form
Functional group	$-SO_2-N(Na)Cl$
The concentration of immobilized active chlorine (% w/w)	5.2 and 9.4
Bulk density (g/mL)	0.80–0.85
Humidity of air-dry product (%)	10.0
Water absorption (%)	150
The average size of the granules (mm)	0.5

General experimental procedure for studying the disinfectant capacity of chlorine-active polymers and the dynamics of chlorine release into a model solution

For the experiment, a daily culture of selected microorganisms was prepared, cultivated in Mueller-Hinton agar. Using a sterile syringe, the culture was administered into 150 mL of commercially available 0.9% sodium chloride solution for pharmaceutical purposes (YURiA-PHARM, Ukraine). This solvent did not contain microorganisms and their metabolites, and its pH of 6.3–7.1 and the absence of hardness salts did not disrupt the structure of the functional group of the polymer. The dilution was carried out so that the concentration of colony-forming units (CFUs) in the resulting model solution was 1.5×10^8 or 1.5×10^6 CFU/mL (by McFarland, densitometer Biosan, Latvia). Then, an accurate weighed portion of the polymer was added to the solution, the flask was placed on an orbital shaker (Heathrow Scientific, USA) and stirred at a given variable speed, periodically taking samples for analysis for 100 min. Sampling was carried out as follows: at certain time intervals, the shaker was stopped for 1 minute to allow the sedimentation of possible polymer microparticles. Using a sterile pipette, 1 mL of the solution was taken, and 1 mL of sterile isotonic NaCl solution was immediately added to the flask to compensate for the decrease in the volume of the model solution, after which the stirring was resumed. The collected 1 mL of the solution was divided into two parts of 0.5 mL each, which were used for two parallel analyses.

A minimum (about 0.01 mL) amount of 0.1 N sodium thiosulfate solution was added to the first part of the water sample to neutralize the chlorine-active species present, and after 3 min, this solution was inoculated onto the Petri dishes with appropriate nutrient medium. To ensure subsequent correct counting of CFUs, at least two decimal dilutions were performed in parallel. The inoculated dishes were incubated for 24–36 h at 37 °C, after which the grown colonies were counted, and the effectiveness of the disinfection procedure was determined by comparing their number on the dishes with the treated and initial solutions. The results are presented as percent microbial reduction, which was calculated using the formula:

$$\text{Microbial reduction } (\%) = \frac{\text{TMC}_{\text{before treatment}} - \text{TMC}_{\text{after treatment}}}{\text{TMC}_{\text{before treatment}}} \times 100\%,$$

where $\text{TMC}_{\text{before treatment}}$ and $\text{TMC}_{\text{after treatment}}$ are total microbial counts in the initial model solution and in the treated solution, respectively.

The remaining 0.5 mL of the collected model solution sample was diluted to 3.5 mL with distilled water and used to determine the concentration of total chlorine, which was carried out colorimetrically using the standard method based on the oxidation of N,N-diethyl-p-phenylenediamine (DPD) with chlorine species (Carlsson *et al.* 1999). Here and further, total chlorine is understood to be the sum of free (hypochlorous acid and hypochlorite ion) and combined (in the form of various chloramines) chlorine. After 100 min, the microbiological analysis was stopped, the solution was stirred for another 2 h, and the total chlorine in it was determined. Then, the solution was left without stirring in a dark place for 24 h, after which the final analysis for total chlorine was carried out. After this, 0.5 g of taurine (Qianjiang Yongan Pharmaceutical Co., Ltd, China) was added to the solution to activate the chlorine release from the polymer and stirred until a constant total chlorine concentration in the solution was established, using it to calculate the residual amount of active chlorine immobilized on the polymer (Murashevych *et al.* 2023). To test the polymer efficiency during repeated use, the granules were immediately filtered after achieving complete disinfection of the model solution, thoroughly rinsed with sterile isotonic sodium chloride solution, and, without drying, quantitatively transferred to the next sample of the same microbial suspension, repeating this operation several times and analyzing the treatment process according to the above-described methods. To determine the possibility of polymer regeneration, in separate experiments, granules after treating with an excess of taurine and no longer containing immobilized chlorine were filtered, rinsed with distilled water, and added to 50 mL of 1,000 mg/l NaOCl solution overnight. Then, the granules were filtered again, repeatedly rinsed with a sterile sodium chloride solution, and, without drying, quantitatively added to the model contaminated solution, studying it according to the general procedure.

Treatment of the water sample with NaOCl for comparative testing

A calculated amount of the commercially available preparation SEKOBREN (UKRTEC KO, Ukraine), which is a highly pure electrochemically generated NaOCl solution manufactured using a special technology (Girenko *et al.* 2023), was added to 150 mL of the model water sample via sterile syringe. The concentration of the preparation was determined immediately before the experiment by iodometric titration. The mixture was stirred on an orbital shaker, and the determination of the microbial count and total chlorine concentration was carried out according to the general procedure described above.

Study of the influence of the organic activator on the disinfection efficiency

The rapid interaction of active chlorine with organic amines suggested the possibility of accelerating its transfer from the polymer to the solution by adding a suitable activator to the water sample, which could lead to changes in the characteristics of the disinfection process. To test this hypothesis, the solution of 1.5×10^8 CFU/mL *S. aureus* ATCC 29213 and 0.01 g of organic aminosulfonic acid taurine (Qianjiang Yongan Pharmaceutical Co., Ltd, China) were added to 0.1 g of the studied polymer (5.2% immobilized active chlorine). The mixture was stirred at 200 rpm and analyzed as described above.

Study of the disinfection process of water from a natural source

To determine the effectiveness of the developed process in relation to polycultures, a water sample was taken from the river Zhabokryach, which flows through the center of the right-bank part of Dnipro city through a system of collectors and is not subject to any purification procedures. The sample was taken in June from a depth of about 10 cm from the surface, 2 m from the bank, at an ambient temperature of 28 °C and a water temperature at the sampling site of 18 °C. The acidity, electrical conductivity, hardness, and dry residue were determined by standard methods. The isolation and identification of microorganisms present in it was carried out on the basis of tinctorial microscopic features and biochemical properties, and complete species identification was carried out based on biochemical indicators using the ID 32C test system (BioMérieux, France). Primary inoculation was carried out on several nutrient media: 5% blood, chocolate, mannitol-salt agar, Endo medium, thioglycollate medium, cetrimide agar, tryptone soy agar (TSA), tryptone soy broth (TSB), and Sabouraud agar. When studying the disinfection process, a polymer was added directly to 150 mL of the water sample, and then, following the above-described general procedure. To calculate the microbial reduction, the culture was performed on meat-peptone agar, thus determining the total microbial count. In parallel, specific nutrient media were inoculated to monitor the presence of each individual type of microorganism, but separate colony counts were not performed.

A polymer sample with immobilized sulfamide groups $-SO_2-NHNa$, which did not contain chlorine, was used as a control in all experiments.

All experiments were repeated at least in triplicate. For each of them, mean values and standard deviations were calculated. The tables and figures below present the mean values of the obtained quantitative characteristics.

RESULTS AND DISCUSSION

Disinfection activity of polymer samples

The dynamics of the reduction of the microbial count in model solutions treated with 0.1 g of polymer sample with 5.2% immobilized active chlorine at a stirring speed of 200 rpm is shown in Figure 1. Full details of the model solutions processing are provided in Supplementary Tables S1 and S2. The relative standard deviation in repeating the experiments did not exceed 9% in any case. Control polymer samples that did not contain immobilized chlorine had no antimicrobial activity.

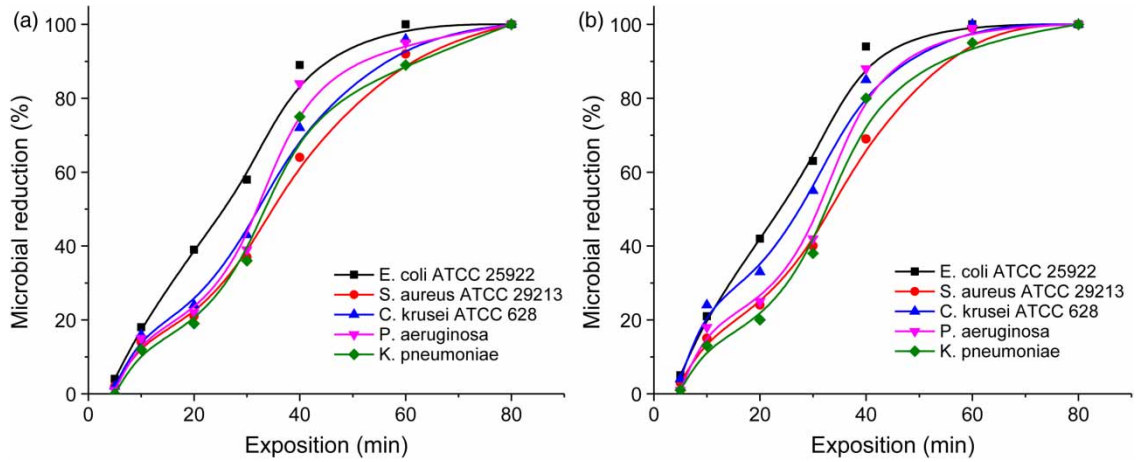


Figure 1 | Dynamics of microbial reduction in model solutions treated with 0.1 g of polymer sample with 5.2% immobilized active chlorine: (a) initial microbial load 1.5×10^8 CFU/mL and (b) initial microbial load 1.5×10^6 CFU/mL.

As seen from Figure 1, the polymer sample revealed pronounced antimicrobial properties. Complete suppression of microorganisms was achieved in all cases within 60–80 min. The decrease in the microbial count proceeds gradually. The *S. aureus* ATCC 29213 demonstrated greater resistance, which has been noted many times before (Bolton *et al.* 1988; Parvin *et al.* 2023) and is associated with the structural features of its cell wall. *K. pneumonae* also exhibits increased resistance, having an additional protective barrier in the form of a polysaccharide extracellular capsule (Rendueles 2020). The least resistant to the procedure was *E. coli ATCC* 25922, which was not detected in any of the solutions after 60 min. Considering that bacteria of this species are most often found in water sources, are associated with numerous negative health effects, and are one of the indicators of water quality (Edberg *et al.* 2000), their high sensitivity to the polymers under study is of great importance for the practical application of the latter.

No significant difference between the antimicrobial efficiency of polymers with 5.2 and 9.4% immobilized active chlorine was observed in the same experimental conditions. At the same time, there was a pronounced tendency to accelerate the disinfection process with an increase in the mass of the polymer used (Figure 2).

This is obviously due to the corresponding increase in the interphase surface, which ensures more effective contact of the microbial cell with the polymer granule and, as will be shown below, leads to a significant increase in the chlorine release into the solution. But, as seen from Figure 2, there is no multiple increase in the antimicrobial effect, which indicates that the key influence on it is collision frequency between a granule and a microbe or metabolite molecule, and not the total amount of immobilized active chlorine added into the solution. The same can be extended to the microbial concentration of the model solution: complete neutralization of microorganisms occurs faster at their initial count of 1.5×10^6 CFU/mL than at a higher concentration of 1.5×10^8 CFU/mL, but no sharp change in rate is noted either.

In general, the results indicate that the key factors influencing the disinfection rate under the conditions studied are the polymer/model solution interphase surface area and the type of microorganism. At the same time, no fundamental differences in the dynamics of the antimicrobial effect were noted between all experiments.

General patterns of chlorine emission from polymers into model solutions

Data on the dynamics of changes in the total chlorine concentration in model solutions during treatment with 0.1 g of polymer sample at a stirring speed of 200 rpm are shown in Figure 3. Supplementary Tables S3 and S4 contain the full data regarding this process. Repeated experiments showed satisfactory convergence, and the deviation of results in no case exceeded 12%, demonstrating a tendency to increase with increasing microbial load, especially at maximum active chlorine concentrations in solutions.

As seen from Figure 3, the presence of microorganisms in all cases provokes a gradually occurring release of chlorine from the polymer to the solution. At the initial stage, which in all cases lasts approximately 60–80 min, an almost linear increase in the total chlorine concentration is observed. Then the gradient of concentration growth decreases, and it reaches the maximum value. There are no significant differences in the rate of reaching this maximum concentration depending on the type of

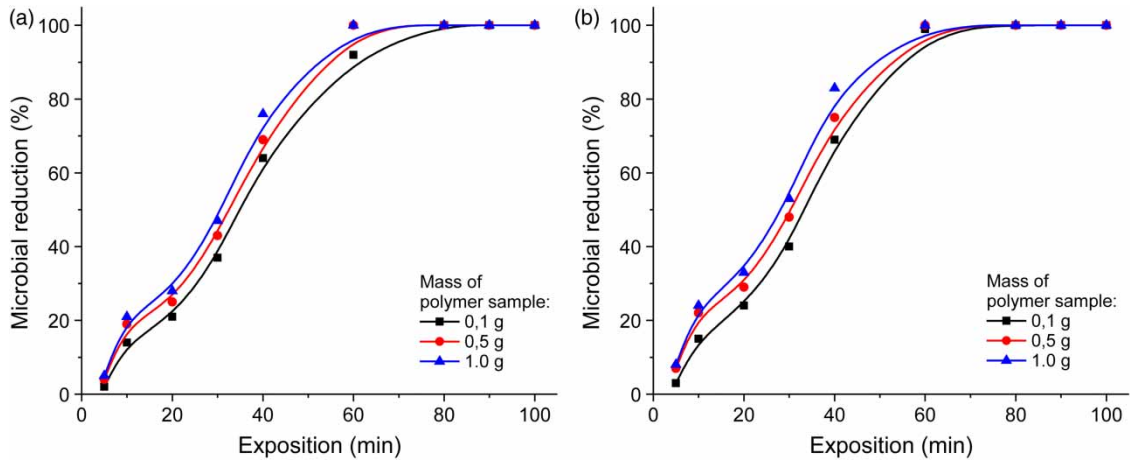


Figure 2 | Dependence of the microbial reduction on the mass of polymer sample (5.2% of immobilized active chlorine) during the treatment of a model solution of *E. coli* ATCC 25922: (a) initial microbial load 1.5×10^8 CFU/mL and (b) initial microbial load 1.5×10^6 CFU/mL.

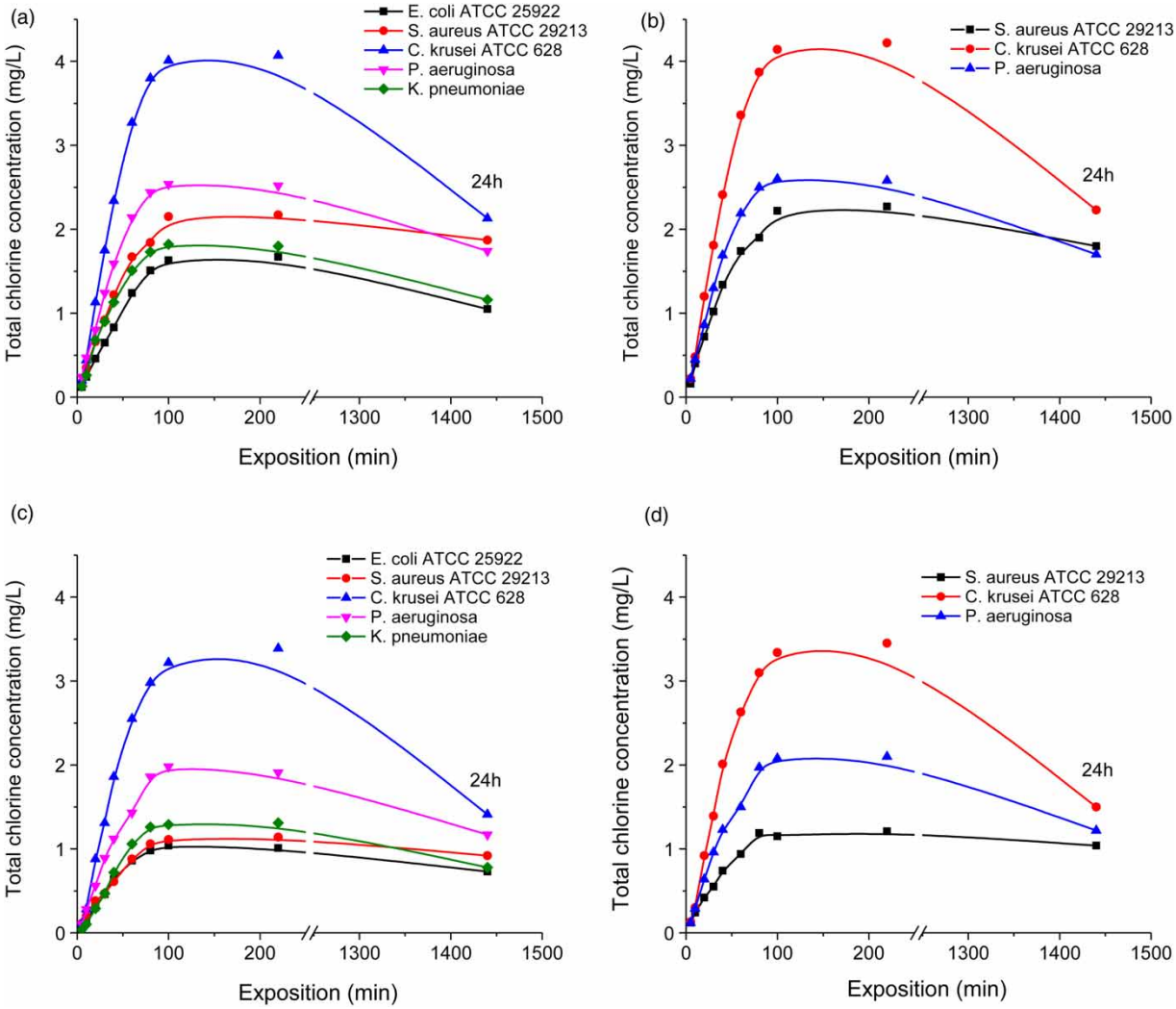


Figure 3 | Dynamics of chlorine release from 0.1 g of polymer sample during the treatment of model solutions: (a) concentration of immobilized chlorine 5.2%, initial microbial load of the solution 1.5×10^8 CFU/mL; (b) concentration of immobilized chlorine 9.4%, initial microbial load of the solution 1.5×10^8 CFU/mL; (c) concentration of immobilized chlorine 5.2%, initial microbial load of the solution 1.5×10^6 CFU/mL; and (d) concentration of immobilized chlorine 9.4%, initial microbial load of the solution 1.5×10^6 CFU/mL.

microorganism. Note that these data correlate with the rate of microbial suppression (Figure 2), which is also fully achieved after 80 min of treatment. The maximum total chlorine concentration itself is determined by the type of microorganism and the microbial load of the model solution. As we wrote earlier, the mechanism of action of the polymer is that when a microbial cell and/or its metabolites come into contact with the functional *N*-chlorosulfonamide groups of the sample, chlorination of the nucleophilic centers occurs, the most active of which are probably the nitrogen atoms of the amino and amide groups of biomolecules. As a result, various *N*-chlorine derivatives are formed, which are detected in the solution as total chlorine. This is evidenced by the higher maximum total chlorine concentration in solutions of one test culture with a higher microbial load, while this concentration itself does not depend on the amount of chlorine immobilized on the polymer. The different influence of the type of microorganism on the chlorine release is also clearly expressed and is determined, apparently, by the different amino acid composition of the microbial cell and, accordingly, the different kinetics of formation and decay of *N*-chloramines formed during its contact with the polymer. Thus, Figure 3 shows that the highest chlorine emission is caused by *C. krusei* ATCC 628, which is explained by the eukaryotic structure of this cell, that is, the presence of a larger number of amino acid residues in the membrane organelles, which are absent in other test cultures. The different properties of the resulting chlorine derivatives also explain the different rates of total chlorine decomposition during storage of

solutions for 24 h after treatment. In the case of *C. krusei* ATCC 628, the concentration of chlorine-active compounds per day decreases more than 2-fold, and for *S. aureus* ATCC 29213, only by 1.2 times. The complexity of the chemical composition and the species-specific features of the microbial cell do not allow predicting the limit of chlorine release into the solution and the stability of the compounds formed. These parameters can only be determined experimentally. However, as seen from Figure 3, even with an initial microbial load of 1.5×10^8 CFU/mL, which corresponds to extremely contaminated water, in none of the cases was more than 5 mg/l of total chlorine detected by the time of complete disinfection. All residual chlorine concentrations obtained were within the ranges of 0.5–5.0 mg/l recommended by WHO.

The dependence of the total chlorine concentration in the solution on the weight of the polymer sample is shown in Figure 4.

As seen, when using 0.5 g of polymer, the chlorine release is more intense than in the case of 0.1 g of the same sample, and a higher (by approximately 20%) maximum concentration is achieved. When moving from 0.5 to 1.0 g, such differences are practically not observed. This effect confirms that the release of chlorine into the solution mainly occurs upon direct contact of the microorganism with the polymer, the efficiency of which depends on the surface area of the granules, the surface concentration of *N*-chlorosulfonamide groups and the diffusion rate of the activator – the microbial cell or its metabolite – to the granule, which is determined by the hydrodynamic mode of the process. This also explains the absence of significant differences in this process for polymers with different contents of immobilized active chlorine (Figure 4). The method of polymer synthesis (Murashevych *et al.* 2024a) implies a practically identical surface concentration of $–SO_2–NClNa$ groups, which corresponds to the maximum achievable degree of conversion of raw carrier polymer. The differences in the total concentration of immobilized chlorine between the two samples used are due to the filling of the deeper layers of the granule. The results obtained show that this is not of decisive importance in the heterogeneous emission process, at least for the studied exposure time. It can be assumed that after all surface functional groups have been used up, for example, when treating large quantities of water or when reusing the polymer, the chlorine release will slow down. This aspect requires a separate study.

The influence of the hydrodynamic mode on the disinfection efficiency

Considering the importance of the heterogeneous component of the process, an experiment was conducted in which a model solution of 1.5×10^6 CFU/mL *E. coli* ATCC 25922 was treated with 0.1 g of polymer containing 5.2% active chlorine and analyzed in different hydrodynamic modes: at 200 rpm, at 100 rpm, and without stirring. The results of the experiment are presented in Figure 5.

As seen, the stirring conditions significantly affect both the chlorine release process and the rate of antimicrobial action, which confirms the key importance of the frequency of contact of the microbial cell or its metabolites with the polymer granule surface. When the stirring speed is reduced from 200 to 100 rpm, the time of complete bacteria suppression increases by approximately 80 min. At the same time, the maximum chlorine concentration is almost the same in both cases, although the

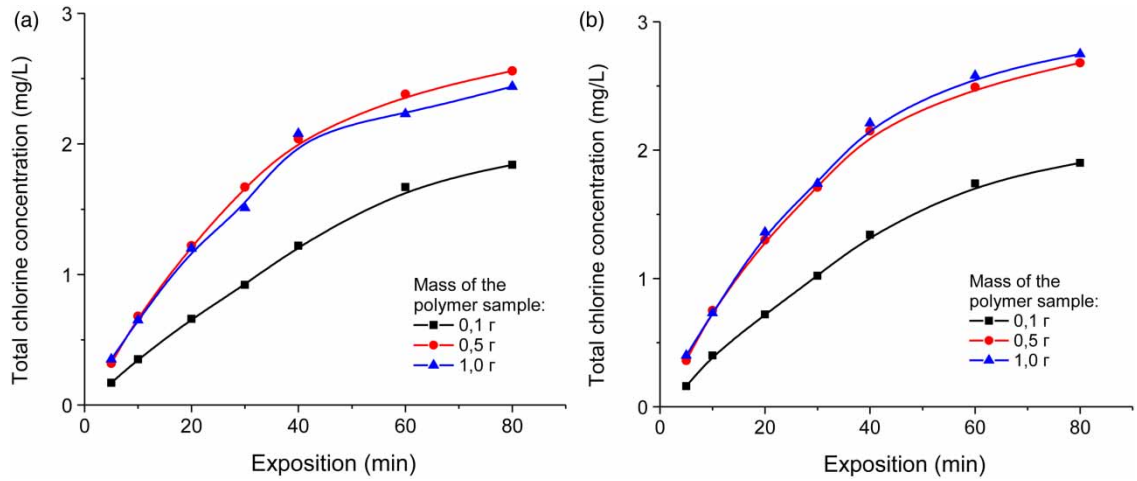


Figure 4 | Dependence of the total chlorine concentration on the mass of chlorine-active polymer during the treatment of the model solution of 1.5×10^8 CFU/mL *S. aureus* ATCC 29213 at 200 rpm: (a) 5.2% of immobilized active chlorine and (b) 9.4% of immobilized active chlorine.

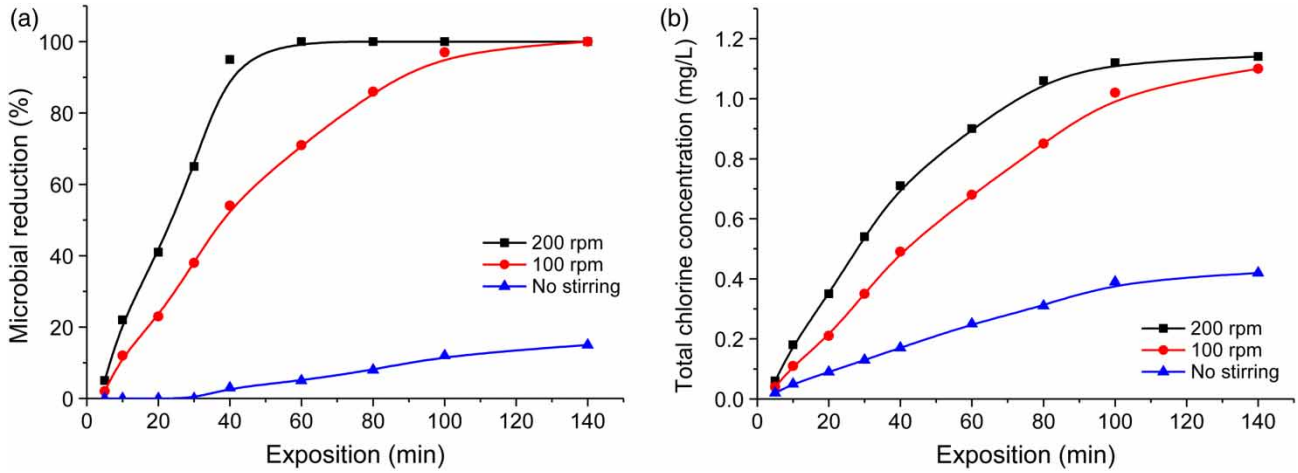


Figure 5 | Dynamics of changes in the parameters of a model solution of 1.5×10^6 CFU/mL *E. coli* ATCC 25922 during treatment at different stirring speed: (a) dynamics of microbial reduction and (b) dynamics of chlorine release.

time to achieve it increases as well. Without stirring, the antimicrobial effect develops extremely slowly, and complete suppression is achieved only on the second day. In the control experiment, by this time, the microbial reduction was approximately 25% due to natural causes (lack of nutrient medium). Nevertheless, our experiment shows that water disinfection via the studied polymers can be, to a certain extent, achieved even under static conditions.

Stability of polymer functional groups and regeneration capacity during water disinfection

In the context of water treatment, an important aspect is the stability of the polymer functional groups during their long-term presence in a contaminated solution. We determined the residual concentration of active chlorine immobilized on 0.1 g of polymer after the granules were kept in treated solutions of different microbiological composition for 24 h. The decrease in this parameter was not statistically different for samples with an initial concentration of 5.2 and 9.4%, and depended on the nature and concentration of microorganisms in the model solution. On average, 0.6–0.9 and 0.3–0.5 mg of immobilized chlorine were consumed to treat 150 mL of a solution with a concentration of 1.5×10^8 and 1.5×10^6 CFU/mL, respectively. The highest values were observed in the case of *C. krusei* ATCC 628. This is somewhat higher than could be assumed based on the maximum achieved total chlorine concentration in the solution during the experiment (Figure 3). Apparently, the rate of decomposition of some *N*-chloramines formed during the experiment is so high that the chlorine transferred from the polymer to the organic molecule is no longer determined as free/combined by the time the solution sample is taken. It is important that during further storage of polymer granules in an already disinfected model solution, the concentration of active chlorine immobilized on them practically does not decrease. Thus, the concentration differed by less than 3% after 1 day and after 10 days in a solution of 1.5×10^8 CFU/mL *C. krusei* ATCC 628. It follows from this that the presence of microbial cell decomposition products does not cause further chlorine release, which significantly increases the service life of the polymer.

The possibility of multiple use of the polymer and its regeneration were studied by treating a suspension of 1.5×10^8 CFU/mL *E. coli* ATCC 25922 with 0.1 g of granules containing 5.2% immobilized chlorine. It was found that with its sequential administration into three portions of the model solution, statistically significant differences were absent both in the rate of achieving complete disinfection and in the maximum concentration of total chlorine accumulated in the solution. This indicates that, in the swollen granules, not only surface but also partially deeper functional groups are involved in the process, without reducing the performance characteristics. In addition, the polymer, having been treated three times with an excess of taurine followed by 'recharging' in a NaOCl solution, demonstrated a return to the initial concentration of active groups and preservation of all parameters of the disinfection process. The resource of such materials over a longer distance and in more aggressive conditions requires additional research.

Comparison of the efficiency of the developed procedure with the NaOCl treatment

The procedure of water disinfection via studied polymers can be contrasted with the classical process of its treatment with a sodium hypochlorite solution. In a comparative experiment, 150 mL of a model solution of 1.5×10^8 CFU/mL *E. coli* ATCC 25922 was supplemented with such an amount of 1,010 mg/l NaOCl solution that its concentration was 2.5 mg/l (in terms of active chlorine), which corresponds to the total chlorine maximum concentration in the solution when using a chlorine-active polymer under the same conditions (Figure 4). The solution was stirred at 200 rpm, and samples were collected in the same way as in the above experiments. The data obtained are presented in Figure 6.

As seen, complete suppression of microorganisms is achieved after 40 min of treatment, which is generally faster than when using polymers, and is due to the homogeneity of the system and the absence of a diffusion component of the disinfection process. At the same time, already within 5 min, the concentration of total chlorine drops by 40%, and after 100 min, by almost 80%. After 24 h, less than 5% of the starting amount of active chlorine remained in the treated solution. Such a change in the concentration of chlorine-active compounds is the opposite of that obtained when treating the solution with polymers. This is explained by the fact that in this case, the oxidation processes of microbial components with sodium hypochlorite, which has a higher oxidation–reduction potential than *N*-chlorosulfonamide, prevail over the chlorination/transchlorination processes with the formation of various *N*-chloramines. Accordingly, the active chlorine consumption is much greater in this case. Rapid decomposition of free chlorine in the presence of an organic load is a known fact, which, among other things, causes a decrease in the effectiveness of hypochlorites in disinfecting water with such impurities (Kwio-Tamale & Onyutha 2024). Also, NaOCl is generally less stable, especially in the light. Thus, on the one hand, the use of NaOCl allows for faster killing of microorganisms. On the other hand, its rapid decay does not provide further protection of water from microorganisms that may enter it after treatment, while using polymers, such protection will be achieved due to the possibility of releasing a new portion of chlorine into the solution.

The influence of taurine on the disinfection efficiency

We have previously shown that the presence of amine impurities of various structures in water leads to a sharp release of active chlorine from the polymer granule with the formation of corresponding *N*-chlorine derivatives, which have their own antimicrobial activity, in solution (Murashevych *et al.* 2021). In the context of the potential use of polymers for water disinfection in the field, the time factor plays a key role, and accelerated chlorine transfer can contribute to increasing the rate of this process. At the same time, when choosing an amine activator, it is important that it is accessible and stable, and the products of its chlorination are non-toxic and do not significantly change the consumer qualities of water. These requirements are met by taurine (2-aminoethanesulfonic acid), which is one of the most common non-proteinogenic

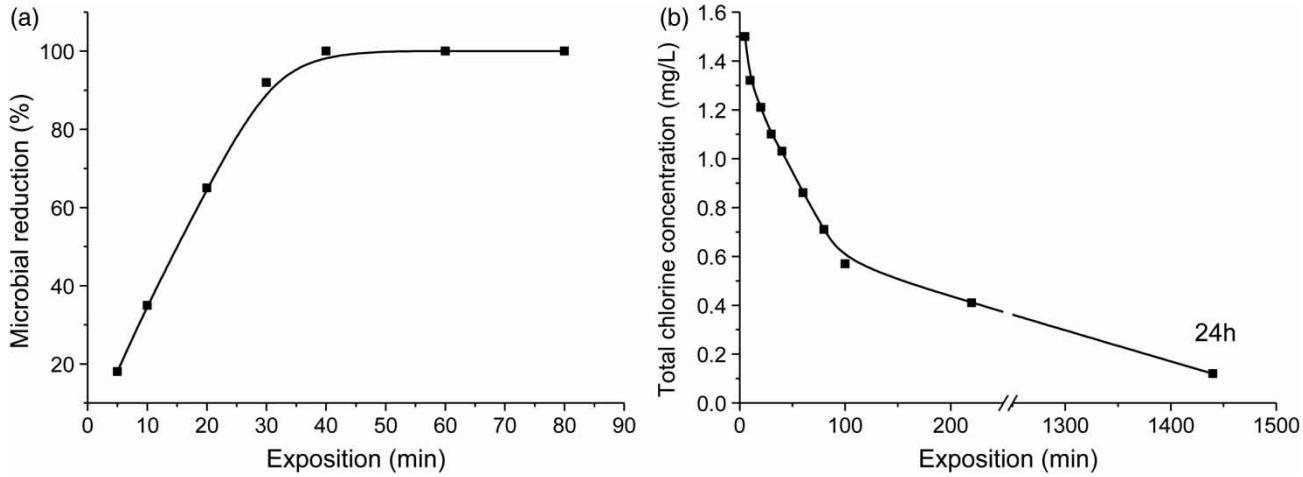


Figure 6 | Results of the treatment of model solution of 1.5×10^8 CFU/mL *E. coli* ATCC 25922 with NaOCl solution: (a) dynamics of microbial reduction and (b) dynamics of chlorine release.

amino acids in the body, has a number of positive pharmacological effects, and is widely used in the food industry (Schaffer & Kim 2018). The product of its interaction with active chlorine is *N*-chlorotaurine, a compound studied in detail by Nagl and colleagues (Gottardi & Nagl 2010; Leiter *et al.* 2020), possessing pronounced antimicrobial properties along with outstanding tolerability. The possibility of obtaining highly pure *N*-chlorotaurine solutions using chlorine-active polymers was described by us earlier (Murashevych *et al.* 2024b).

The results of determining the dynamics of changes in the microbial count and the total chlorine concentration in the model solution of 1.5×10^8 CFU/mL *S. aureus* ATCC 29213 during its treatment with 0.1 g of polymer under taurine activation are presented in Figure 7.

Figure 7 shows that the total chlorine concentration in the solution increases significantly faster and reaches much higher values than without taurine (Figure 4). As expected, this is due to the rapid formation of *N*-chlorotaurine, the presence of which is confirmed by the UV spectrum of the resulting solution with a characteristic peak at 251 nm. A higher (compared with the microbial amino groups) concentration of the activator leads to the polymer chlorinating predominantly this molecule. Further, the main process probably becomes transchlorination between *N*-chlorotaurine and microbial amino components. However, the antimicrobial effect in this case is much less pronounced, and even after 24 h, viable microorganisms are still present in the solution. This indicates that, despite the previously described microbicidal properties of *N*-chlorotaurine, under the studied conditions, its oxidizing and chlorinating properties are insufficient.

Disinfection of water from a natural source with chlorine-active polymers

Microbiological analysis showed that the water sample taken from the river Zhabokryach contains the following microorganisms: *Bacillus* spp., *Candida* spp., *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Sarcina* spp., *Actinomycetes* spp., and *Aspergillus* spp. The total microbial count in it was 3.0×10^4 CFU/mL. The water also contained a significant amount of dissolved salts: hardness was 152 ppm (as $CaCO_3$), electrical conductivity was 1,120 μs/cm, and pH was 7.3. The high dry residue of 1,885 mg/l at this level of electrical conductivity suggests a significant content of organic impurities.

The sample was treated with 0.1 g of polymer (5.2% immobilized active chlorine) at 200 rpm. The experimental results are illustrated in Figure 8.

The data indicate that a positive effect was achieved in this case as well. Note that the rate of chlorine emission and its maximum concentration were higher than could be predicted based on the initial microbial load, extrapolating these values to those for the model solution of 1.5×10^6 CFU/mL (Figure 4). This is probably due to the presence of non-microbiological amine-containing or ammonium compounds in water, able to provoke additional release of chlorine. Complete suppression of microorganisms was achieved within 40 min. It was noted that the first (by the 20th minute) to neutralize

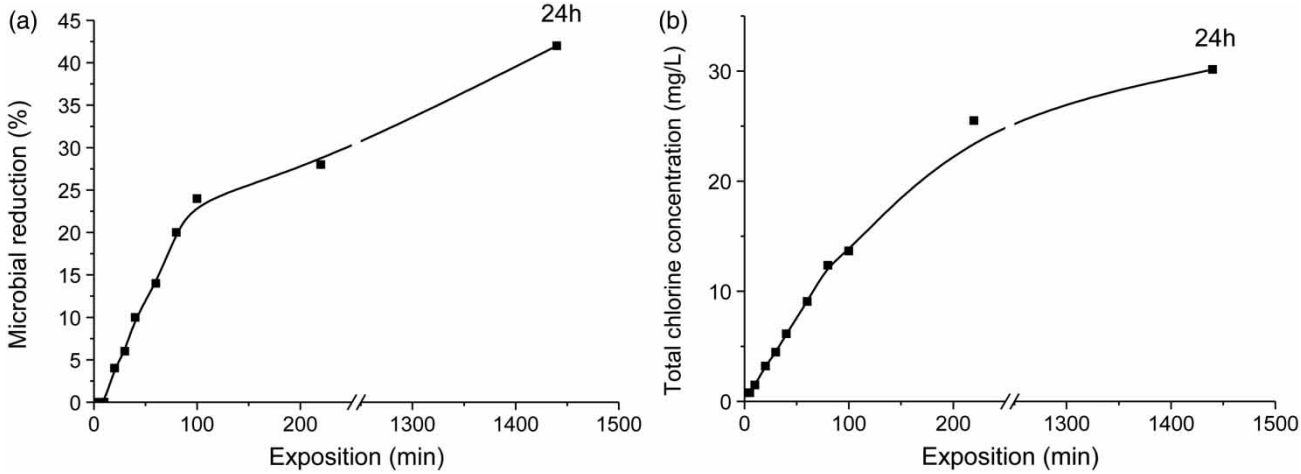


Figure 7 | Results of the treatment of model solution of 1.5×10^8 CFU/mL *S. aureus* ATCC 29213 with 0.1 g of chlorine-active polymer in the presence of taurine: (a) dynamics of microbial reduction and (b) dynamics of chlorine release.

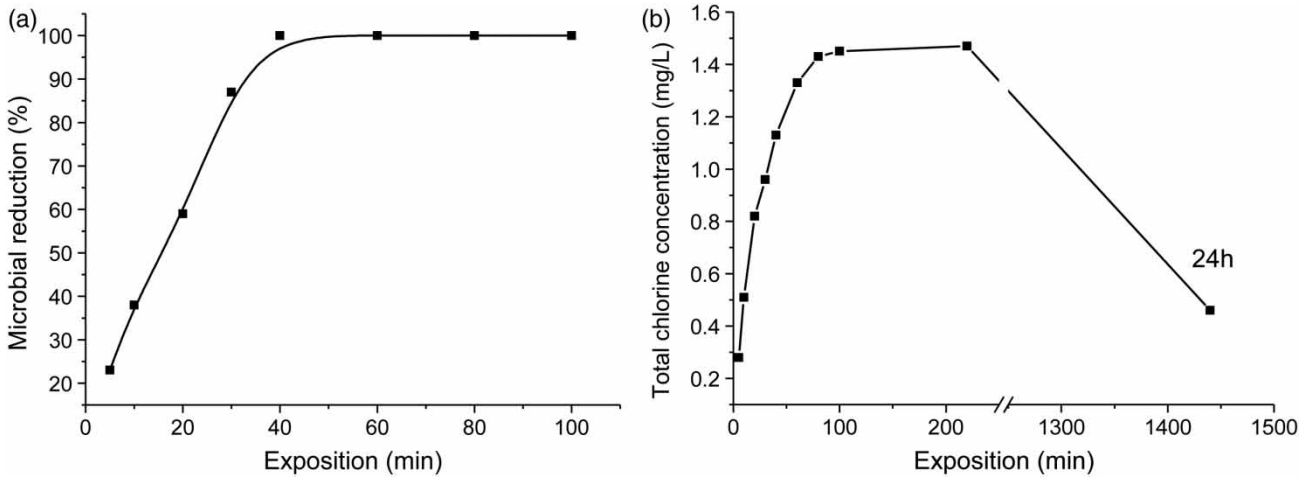


Figure 8 | Results of the treatment of a water sample from a natural source with chlorine-active polymer: (a) dynamics of microbial reduction and (b) dynamics of chlorine release.

were representatives of the *Enterobacteriaceae* family, and microorganisms of the *Bacillus* family were the most resistant. Also noteworthy is the significant, more than 3-fold, decrease in the concentration of total chlorine after 24 h, which can also be explained by the presence of chemical impurities in the water that form unstable *N*-chloramines or are capable of oxidation. The lack of accurate data on the organic matter content in the sample can be attributed to the shortcomings of our study.

To summarize, it can be concluded that synthesized chlorine-active polymers have broad prospects in water treatment technologies. The efficiency of similar materials was shown in earlier studies (Bogochek & Kotsiolek-Balyaveider 1987; Emerson 1990), but in our work, correlations were made between the rate of disinfection and the accumulation of chlorine compounds in the water sample. The main advantage of the proposed disinfection method is that the release of active chlorine from the polymer is proportional to the degree of water pollution, which reduces the risk of its overdose. Considering the much lower oxidation–reduction and chlorinating potential of *N*-chlorosulfonamides, we can assume a lower probability of the appearance of toxic organochlorine compounds in the treated water in comparison with the classic chlorination with sodium hypochlorite. At the same time, the polymer itself does not emit any compounds into the treated water except active chlorine, and can be easily extracted, used repeatedly, and regenerated (Murashevych *et al.* 2024a). Polymer granules are compact, stable during storage, and the method of their application is simple and potentially can be carried out in the field.

The shortcomings of this study include the lack of data on the suppression of more typical waterborne microorganisms, such as various Salmonella, coliform bacteria, etc.; also, the effectiveness of such treatment against viruses was not studied, although the virucidal properties of the polymers used were shown by us earlier (Murashevych *et al.* 2022). The nature of chlorine-active compounds formed in model solutions was not analyzed. In further research, it seems promising to study the process of water treatment with synthesized polymers in flow systems, determine their ion-exchange properties, and also consider the possibility of their combinations with other functional materials (adsorbents, activators, catalysts for the active chlorine decomposition, etc.) to create compact devices for on-site complex water purification.

CONCLUSIONS

The conducted studies have shown that the granular polymers with immobilized *N*-chlorosulfonamide groups demonstrate pronounced antimicrobial properties when immersed in contaminated water environments. They are capable of neutralizing mono- and polycomponent bacterial suspensions, including those containing multi-resistant strains, in a wide range of microbial loads. The effect is achieved due to the transfer of active chlorine from the polymer into the solution upon contact of the granule with the microbial cell or its metabolites. The disinfection rate mainly depends on the hydrodynamic mode of the process and the phase interface, with certain species-dependent features. The accumulation of total chlorine compounds

in the solution is proportional to the microbial load of the latter, and also apparently depends on the number of functional groups of biomolecules available for chlorination, which is proven by the most intense increase in chlorine concentration during the treatment of a suspension of eukaryotic *C. krusei* ATCC 628. At the same time, even the treatment of the water sample with a very high microbial concentration of 1.5×10^8 CFU/mL, by the time of complete disinfection, did not in any case lead to an excess of the total chlorine content above the threshold of 5 mg/l recommended by WHO. The stability of the polymer functional groups when immersed in microbe-containing solutions indicates a potentially high resource of their work, during which the polymer is able to prevent further contamination. Such properties of polymers, together with their compactness, stability, and the possibility of regeneration, make them promising for use in water treatment systems, including in the field.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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