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Impact of selected activators and inhibitors on efficiency in removing haloacetic acids from water in a reactor with native and immobilised biocatalysts

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ABSTRACT

The objective of the described tests was to determine the impact of selected activators and inhibitors on the efficiency of removing mixtures of haloacetic acids (HAAs) from water. The tests were conducted in the bioreactor equipped with an ultrafiltration polyacrylonitrile membrane with native and immoblised enzymes. The scope of tests included three series of measurements for reference waters, where each acid (monochloroacetic acid—MCAA, dichloroacetic acid—DCAA, trichloroacetic acid—TCAA, monobromoacetic acid—MBAA, and dibromoacetic acid—DBAA) was mixed with another acid. The mixtures with HAA concentrations within the range from 0.01 to 0.005 mmol/dm^3 varied in their qualitative and quantitative composition. The obtained test results led to the conclusions that three of five tested acids were competitive inhibitors—MCAA and MBAA for DCAA, TCAA and DBAA, and DCAA for TCAA and DBAA. Such a regularity was observed for both native and immobilised enzymes. The catalytic activity of native enzymes was higher by ca. 38%, compared to immobilised enzymes. However, immobilised biocatalysts were far less prone to the impact of inhibitors. It was noticed that Cl^-, SO_4^{2-}, Mg^{2+}, Zn^{2+}, Ca^{2+}, and Fe^{3+} ions had no effect on the activity of applied enzymes, both in the case of native and immobilised enzymes. Only a slight increase (by ca. 3–4%) in the catalytic activity of enzymes was observed in both cases in the presence of Mn^{2+}.

Keywords: Haloacetic acids; Native and immobilised enzymes; Biodegradation; Enzymatic activity

1. Introduction

The rapid development of modern biotechnology techniques, the release of individual cellular enzymes, and the identification of new biocatalysts, whose properties have been unknown so far—all of them have made enzymes the core element in developing technological processes. Conventional chemical processes are more and more often replaced by the technologies involving the stable enzyme preparations of specifically defined properties. They are obtained by

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immobilising enzymes on or in stable supports, e.g. polymer membranes. The performance characteristics of those preparations are determined by features of both the support and the enzyme, which reciprocally shape each other as the effect of the applied immobilisation process. A very important advantage of immoblised proteins (compared to free cells) is the fact that they are not washed out and are more resistant to toxic effects of xenobiotics. The current and potential areas of innovative applications of immobilised proteins include medicine, analysis, environmental protection and engineering, food and pharmaceutical industry, and organic synthesis, representing ca. 10% of the total worldwide applications of catalysts. Mastering synthesis processes and applying enzymatic catalysts are becoming more and more crucial for global problems related to ecology, food and energy [1–4].

Haloacetic acids (HAAs) are usually formed during chlorination of water containing their organic precursors. The quantitative content of HAA in TOX (the total amount of halogenated organic compounds) is usually lower than that of trihalomethanes, but it is higher compared to other groups of chlorination by-products. HAA concentrations are directly proportional to the dose of chlorine and the content of organic precursors in water. Nine HAAs have been found in water treated with chlorine. The main representatives of HAA marked with the symbol HAA5 include chloroacetic acid ($CH_2ClCOOH$—MCAA), bromoacetic acid ($CH_2BrCOOH$—MBAA), dichloroacetic acid ($CHCl_2COOH$—DCAA), trichloroacetic acid (CCl_3COOH—TCAA), and dibromoacetic acid ($CHBr_2COOH$—DBAA). Moreover, the following acids can be noticed in purified water: tribromoacetic acid (CBr_3COOH—TBAA), bromochloroacetic acid ($CHBrClCOOH$—BCAA), dibromochloroacetic acid ($CBr_2ClCOOH$—DBCAA), and dichlorobromoacetic acid ($CCl_2BrCOOH$—DCBAA) [5–7].

According to the US Environmental Protection Agency provisions from 2008, the total amount of five HAAs (monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid) should not exceed 60 mg/m^3. However, this value is projected to be reduced to 30 mg/m^3 because HAA has been considered to pose a carcinogenic risk to human and animal health. According to WHO guidelines concerning potable water quality, the acceptable concentration of monochloroacetic acid is 20 mg/m^3, dichloroacetic acid is 50 mg/m^3, and trichloroacetic acid is 200 mg/m^3.

Nowadays, the concentration of HAAs in municipal water can be reduced in three ways: The first method, regarded as the most appropriate way to reduce HAA concentrations, is the removal of HAA precursors. The second method consists in using disinfectants, other than chlorine; and the last one involves the removal of already existing HAAs. The properties of enzymes occurring in living organisms can be applied for eliminating HAA from water. The usability of biocatalysts for those purposes is caused by, *inter alia*, their high specificity of activity which results in performing specifically defined reactions, and their natural origin makes them non-toxic to water purification. Although the formation of enzymes is quite complex, the application costs are relatively low. The advantage of using biocatalysts in the process of removing HAAs from water is the possibility of adjusting their activity and the rate of performed processes by, *inter alia*, controlling the impact of environmental conditions, in particular temperature, pH, and the presence of adequate activators and inhibitors [8–11].

There are many types of molecules (known as inhibitors) which are capable of reducing (disturbing) the catalytical activity of a specific enzyme. Some of them are typical cellular metabolites which inhibit the specific enzyme during the natural metabolic control of the adequate pathway, and others are substances foreign to the organisms, such as toxins and drugs (mainly antibiotics). The enzymatic reaction can be inhibited by denaturation of enzymatic proteins (e.g. a drastic change in pH or temperature, the presence of organic solvents, salts of heavy metals) or under the impact of compounds which irreversibly bound to the enzyme resulting in its deactivation (irreversible inhibition). Reversible inhibition occurs when, after removing the inhibitor, the enzyme recovers (at least partially) its activity [1,12,13].

2. The aim of the study

The tests, whose results are described in this article, are the continuation of previous studies on efficient removal of HAAs from water in the bioreactor with enzymatic ultrafiltration membranes [14]. The studies were aimed at determining the impact of selected inhibitors and activators on the efficiency of HAA removal from water in the bioreactor with native and immobilised enzymes. The enzymatic activity of the applied biocatalyst determined the process efficiency. The practical application of HAA optimal concentrations, experimentally determined, in the mixture (feed solution) and the selection of those acting as inhibitors can result in the total removal of all selected acids from reference water at the same time and to the same degree. This will also provide for predicting

time and the degree of removing particular HAAs from the purified water. The scope of tests presented in this article included the following:

(1) The tests on determining which of the aqueous solutions of five selected HAAs inhibited the biodegradation of HAA, using the native enzymes and the enzymes immobilised on the flat ultrafiltration polyacrylonitrile membrane. Moreover, the effect of inhibitor concentration on the degradation rate of other acids was determined.
(2) The effect of Mg^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Fe^{3+}, Cl^{-}, and SO_4^{2-} ions on the rate of removing the mixture of five HAAs (MCAA, DCAA, TCAA, MBAA, and DBAA) from water in the bioreactor with native and immobilised enzymes was determined as well. In the tests, the concentrations of individual ions in the samples were 0.025, 0.25, 2.5, and 25.0 mmol/l, respectively.

3. Experimental procedure

Enzymes required to degrade haloacetic acids were produced by the specific strain of microorganisms. The strain was isolated from the regular population of activated sludge, which had been firstly undergone methane fermentation and next adapted to the decomposition of HAA. The adaptation of microorganisms was carried out by introducing increasing doses of HAA to the culture.

Microorganisms found to be dominant in the population were *Acinetobacte, Arthrobacter, Pseudomonas, and Bacillius*. All strains used in the study were adapted up to 5 mg/l of each of the investigated acids. Enzymatic fractions were isolated by means of the Hagemann's method.

The analysis of HAAs starts with acidification of a contaminated water sample to pH < 2. Thus, the dissociation of acids is stopped and it is possible to extract them to the organic solvent (liquid-liquid extraction—LLE), which is insoluble in water. The solvent usually used for that purpose is methyltert-butylether (MTBE). The extraction of such polar compounds like HAAs is usually enhanced by salting out. Extracted acids are derivated to methyl esters which are next separated using a gas chromatograph equipped with an electron capture detector. The limit of detection of this technique is usually at the level of 0.5 μg/l, except of monochloroacetic acid for which it is established at 1 μg/l. In this study, the preparation of water sample to analysis was made on the basis of US EPA 552.2 method while the analytical procedure was performed using GC-MS qualitative–quantitative method [15]. The gas chromatograph conjugated with a mass detector (GC-MS), model Saturn 2100T by Varian was used for analytical purposes.

The concentration of the active enzyme was determined using colorimetric Bradford method which relied on the color reaction of the enzyme with Bio-Rad Protein Assay reagent. UV–VIS Cary 50 (by Varian) spectrophotometer was used as a measuring device.

The activity of the enzyme used in the tests as the biocatalyst of protein complex was defined as the quantity of HAAs decomposed during 1 h (expressed as millimoles of acid). While determining the activity, the concentration of solutions of particular acids was 0.01 mmol/l, temperature—25˚C, and pH of acid solutions—2.48 (the optimal conditions for conducting the biodegradation of HAAs determined in the previous tests) [14]. The activity was determined for each of five HAAs. The results for native enzymes are presented in Table 1.

The activity of enzymatic membranes was specified by filtrating through them the solutions of particular acids for one hour. The transmembrane pressure was 0.1 MPa, and the intensity of stirring feed solution was 50 rpm. Then, acid concentration was determined in the particular ultrafiltration streams (feed solution, permeate, retentate). It was the basis for calculating the quantity of each decomposed HAA over time according to the following dependence (1):

$$B_d = 1 - (C_p \times V_p + C_r \times V_r)/C_n \times V_n \times 100\% \quad (1)$$

Table 1
The amount of active enzyme used in the study and its enzymatic acitivity

	The activity of enzyme with regard to the acid (T = 25˚C and pH 2.48) (mmol of acid/1 h) × 10^3				
The amount of the enzyme (mg)	MCAA	DCAA	TCAA	MBAA	DBAA
23.8	5.2151	5.2151	5.2150	5.2151	5.2150

where B_d—the degree of biodegradation of HAA (%), C_p—the concentration of HAA in permeate (mmol/l), C_r—the concentration of HAA in retentate (mmol/l), C_n—the concentration of HAA in feed (mmol/l), V_p—volume of permeate (l), V_r—volume of retentate (l), V_n—volume of feed (l).

The tests on the effect of inhibitors on the rate of ultrafiltration biodegradation of the mixture of selected HAAs in the bioreactor with the flat polyacrylonitrile membrane were performed in the S-76–400 type reactor by Nuclepore. The reactor of 500 cm^3 volume was equipped with a magnetic stirrer. This reactor was able to operate with the flat membrane having the area of 38.5 cm^2. The transmembrane pressure was 0.1 MPa, and the intensity of stirring feed solution was 50 rpm (optimal conditions for conducing the biodegradation of HAAs determined in the previous tests).

The flat, a polyacrylonitrile membrane for ultrafiltration was used as the support for immobilising the active protein. This membrane was obtained by phase inversion of polymer solution containing 17.5% of polyacrylonitrile. The membrane was chemically modified with hydrazine hydrate and glutaraldehyde to create a stable covalent bond between its surface and the enzyme. The enzyme was immobilised on that modified support, and the activity of the obtained membrane (PAN-17,5E) was determined (Table 2).

4. Results and discussion

The analysis of the enzymatic activity of the complex of enzymes used in the tests (Tables 1 and 2) indicated that the removal of all five HAAs at the same level and to the same degree could be expected (apart from the fact that immobilisation reduced the activity of the biocatalyst by ca. 38%, compared to the activity of native enzymes). After one-hour biodegradation, the estimated degree of removing each HAA from the bioreactor with native enzymes should be equal to ca. 57%, however, it was not the case. After one-hour biodegradation of HAA from the mixture of five selected acids (MCAA, DCAA, TCAA, MBAA, and DBAA), MCAA and MBAA were removed by 51%, DCAA was removed by 32%, and TCAA and DBAA were removed by 23%. After two hours, MCAA and MBAA were completely removed, after 2.5 h—DCAA, and after 3 h—other acids (Fig. 1). The similar dependence was observed during the degradation of the mixture of five HAAs in the bioreactor with immobilised enzymes. After 1 h after the onset of the process, MCAA and MBAA were removed by 41%, DCAA—by 25%, TCAA and DBAA—by 20%. After three hours of biodegradation by ultrafiltration, MCAA and MBAA were completely removed, whereas DCAA was removed after 3.5 h, and TCAA and DBAA were removed after 4 h (Fig. 2). The obtained results suggested that MCAA and MBAA behaved like inhibitors for other three acids, and DCAA for TCAA and DBAA. It was decided to prove the above thesis experimentally.

The study on the determination of the inhibiting presence of chosen HAAs on the effectiveness of their removal from water with the used of immobilized enzymes was carried out. One hour long filtrations at 25℃ on PAN-17,5E membranes were made, and 500 cm^3 of feed solutions containing acids mixtures (four filtrations of every of three feeds differ in the type and HAA concentration as well as in the accompanying acid) were performed. The transmembrane pressure was held at the level of 0.1 MPa, and the stirring speed of the feed was equal to 50 rpm. In Tables 3–5, compositions of particular feed solutions (simulated mixtures) and HAA concentrations obtained after one hour of ultrafiltrating biodegradation are presented.

In the first series of the study (Table 3), after 1 h of biodegradation, when the concentration of the basic and the accompanying acid reached the same level, one could clearly notice that MCAA and MBAA acids were favorably removed (the highest removal rates). Their removal efficiency was always ca.25%, while for the rest of the acid it reached 22% for DCAA (in the mixture with MCAA or MBAA) and 23% in the presence of TCAA and DBAA. The average biodegradation rate of TCAA and DBAA (at the presence of MCAA and MBAA) was 20%, 21% in the presence of DCAA and, finally, 25%, when they were mixed with

Table 2
The amount of active enzyme used in the study and enzymatic membrane acitivity

The amount of immobilized enzyme (mg)	The activity of enzyme with regard to the acid (mmol of acid/1 h/38.5 cm^2 of membrane area) × 10^3				
	MCAA	DCAA	TCAA	MBAA	DBAA
23.8	3.2334	3.2334	3.2334	3.2334	3.2334

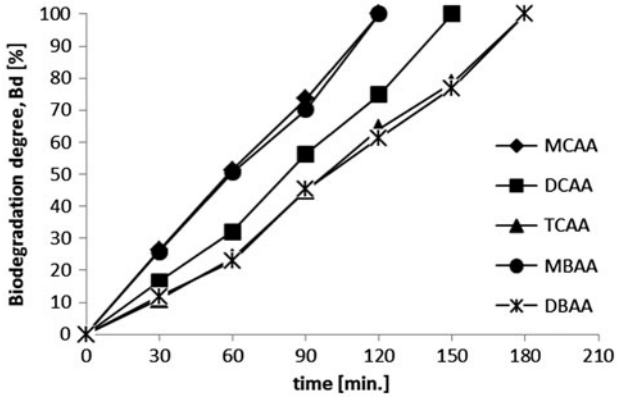

Fig. 1. Dependence of the biodegradation degree of HAAs on duration of process in bioreactor with native enzymes.

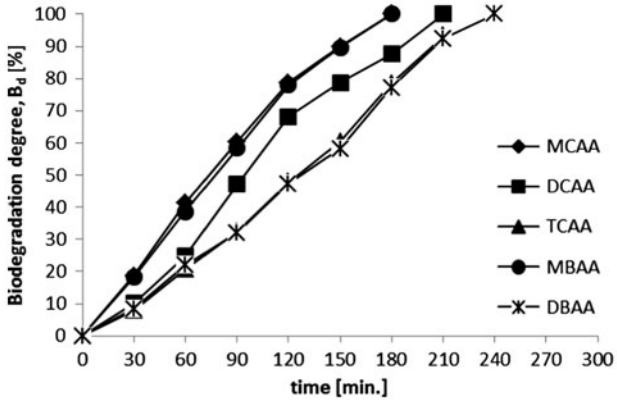

Fig. 2. Dependence of the biodegradation degree of HAAs on duration of process in bioreactor with immobilized enzymes.

each other. The obtained results confirmed the inhibiting character of MCAA and MBAA regarding the rest of acids, while DCAA revealed such properties regarding TCAA and DBAA. In order to show more clearly the inhibiting action of one acid to another, in the second series of the study, the concentration of the basic acid was kept constant (1 mmol/l), while the concentration of the accompanying acid was decreased to the level of 0.75 mmol/l (Table 4).

In the second series of the study (Table 4), after one hour of the biodegradation run, it was observed that the removal rates of monochloroacteic and monobromoacetic acids were still the highest and equal to 25% (regardless of the type of the accompanying acid). The decrease in the inhibitor concentration resulted in an increase in removal efficiency of other acids. Dichloroacetic acid (in the case of being the basic one) was removed in 23% at the presence of MCAA or MBAA (1% B_d increase in refer to the first study series) and in 24% (also 1% removal rate increase), when it was accompanied with TCAA or DBAA. Both trichloroacetic and dibromoacetic acids were removed at the level of 22% (at the presence of MCAA or MBAA) and of 23%, while mixed with dichloroacetic acid. In the case when TCAA and DBAA were undergone to ultrafiltrating biodegradation in the one feed solution where removal rates were the same and equal to 25%.

In the third series of the study (Table 5), the concentration of the base acid was still kept unchanged (1 mmol/l), while the concentration of the accompanying acid was again decreased to the level of 0.5 mmol/l. The lower concentration of inhibitors resulted in the further increase in the removal efficiency of other acids. Such a dependence is observed when competing inhibition mechanism occurs between components. In the third series of the study, biodegradation rates of DCAA, TCAA, and DBAA reached 24%, while ones of MCAA and MBAA stayed at the level of 25% (in case when they acted as the basic acid in the feed).

The obtained results confirmed previous observations that three among five investigated acids were inhibitors i.e. MCAA and MBAA for DCAA, TCAA, and DBAA, while DCAA for TCAA and DBAA. Both monochloroacteic and monobromoacetic acids were removed with the same extent regardless of the accompanying compound in the feed. However, a decrease in their concentration in the feed positively affected the removal efficiency of other HAA. At higher concentrations of substrates, inhibitors (i.e. MCAA and MBAA) competed for the active center of the biocatalyst with the higher amount of substrate particles, and thus the competitive inhibition occurred with the lower intensity.

In Table 6, the average values of biodegradation rates of investigated HAAs obtained during particular series of the performed study are presented.

The study on the impact of the presence of chosen HAAs on the effectiveness of removal of their mixture from water with the use of native enzymes was discussed in the article "The effectiveness of removal of HAAs from water using bioreactor with native enzymes" [16]. Experiment were carried out in flasks of volume 300 cm^3 (also in a configuration of four processes for every of three feed compositions, which were exactly the same as ones used in the study with membrane ultrafiltration bioreactor with immobilized enzymes). 100 cm^3 of the feed was placed in every flask, and 1 cm^3 of enzymes suspension was added. Flasks were thermostated for one hour at 25°C. After this time, the concentration of particular acids was

Table 3
Composition of feed solutions and HAA concentrations at the first series of measurement tests on HAA biodegradation by ultrafiltration

		HAA concentration and biodegradation rates obtained after 1 h of the process			
		Basic acid		Accompanying acid	
Series no.	The feed composition and acids concentration	Concentration (mmol/l) × 10^3	B_d (%)	Concentration (mmol/l) × 10^3	B_d (%)
		DBAA		Accompanying acid	
I	0.01 mmol/l DBAA + 0.01 mmol/l MBAA	8.002	19.98	7.501	24.99
	0.01 mmol/l DBAA + 0.01 mmol/l DCAA	7.914	20.86	7.833	21.67
	0.01 mmol/l DBAA + 0.01 mmol/l TCAA	7.513	24.87	7.508	24.92
	0.01 mmol/l DBAA + 0.01 mmol/l MCAA	8.002	19.98	7.489	25.11
		TCAA		Accompanying acid	
I	0.01 mmol/l TCAA + 0.01 mmol/l MBAA	8.004	19.96	7.499	25.01
	0.01 mmol/l TCAA + 0.01 mmol/l DCAA	7.896	21.04	7.835	21.62
	0.01 mmol/l TCAA + 0.01 mmol/l DBAA	7.513	24.87	7.508	24.92
	0.01 mmol/l TCAA + 0.01 mmol/l MCAA	7.890	20.11	7.491	25.09
		MCAA		Accompanying acid	
I	0.01 mmol/l MCAA + 0.01 mmol/l MBAA	7.489	25.11	7.501	24.99
	0.01 mmol/l MCAA + 0.01 mmol/l DCAA	7.501	24.99	7.816	21.84
	0.01 mmol/l MCAA + 0.01 mmol/l TCAA	7.500	25.00	7.990	20.10
	0.01 mmol/l MCAA + 0.01 mmol/l DBAA	7.487	25.13	8.003	19.97
		MBAA		Accompanying acid	
I	0.01 mmol/l MBAA + 0.01 mmol/l MCAA	7.500	25.00	7.491	25.09
	0.01 mmol/l MBAA + 0.01 mmol/l DCAA	7.501	24.99	7.813	21.87
	0.01 mmol/l MBAA + 0.01 mmol/l TCAA	7.493	25.07	8.002	19.98
	0. mmol/l MBAA + 0. mmol/l DBAA	7.497	25.13	8.002	19.98
		DCAA		Accompanying acid	
I	0.01 mmol/l DCAA + 0.01 mmol/l DBAA	7.733	22.67	7.914	20.86
	0.01 mmol/l DCAA + 0.01 mmol/l TCAA	7.835	22.62	7.914	20.86
	0.01 mmol/l DCAA + 0.01 mmol/l MBAA	7.813	21.87	7.499	25.01
	0.01 mmol/l DCAA + 0.01 mmol/l MCAA	7.813	21.87	7.497	25.13

measured, and the biodegradation rate of every of the acid was determined.

In the first series of the study after one hour of biodegradation, when the concentration of both the basic and the accompanying acid was the same, it was noticed that MCAA and MBAA were always removed the most efficiently. Their biodegradation rate was always ca. 57%. The average biodegradation efficiency of other acids was 52% for DCAA and 45% for TCAA and DBAA. Similarly as in the case of membrane bioreactor studies, it was shown that MCAA and MBAA acted as inhibitors for other acids, while DCAA affected the decomposition of TCAA and DBAA. The results obtained during the first series of the study are shown in Figs. 3–5.

The dependences obtained for MBAA were the same as the ones of MCAA, while the results determined for TCAA corresponded to ones observed for DBAA. In the second series of the study, the removal rate of MCAA and MBAA stayed at the level of 57%; however, the negligible tendency of removal efficiency decrease was observed and it was still the highest in comparison to other acids. The decrease in inhibitors concentration resulted in the increase in decomposition rates of other acids i.e. 52% for DCAA and 49% for TCAA and DBAA.

When the concentration of inhibitors was again decreased (the third study series) the efficiency of removal of other acids improved. The average biodegradation rate of DCAA was 54%, while one of TCAA and DBAA at 52%. The removal rate of MCAA and MBAA was still the highest and equal to 57% (in the case of their role as the basic acid in the feed). The conditions and detailed results of the

Table 4
Composition of feed solutions and HAA concentrations at the second series of measurement tests on HAA biodegradation by ultrafiltration

		HAA concentration and biodegradation rates obtained after 1 h of the process			
		Basic acid		Accompanying acid	
Series no.	The feed composition and acids concentration	Concentration (mmol/l) × 10^3	B_d (%)	Concentration (mmol/l) × 10^3	B_d (%)
		DBAA		Accompanying acid	
II	0.01 mmol/l DBAA + 7.5·10^{-3} mmol/l MBAA	7.806	21.94	5.631	24.93
	0.01 mmol/l DBAA + 7.5·10^{-3} mmol/l DCAA	7.699	23.01	5.762	23.17
	0.01 mmol/l DBAA + 7.5·10^{-3} mmol/l TCAA	7.499	25.01	5.635	24.87
	0.01 mmol/l DBAA + 7.5·10^{-3} mmol/l MCAA	7.782	22.18	5.626	24.98
		TCAA		Accompanying acid	
II	0.01 mmol/l TCAA + 7.5·10^{-3} mmol/l MBAA	7.997	20.03	5.622	25.04
	0.01 mmol/l TCAA + 7.5·10^{-3} mmol/l DCAA	7.893	21.07	5.767	23.11
	0.01 mmol/l TCAA + 7.5·10^{-3} mmol/l DBAA	7.513	24.87	5.624	25.01
	0.01 mmol/l TCAA + 7.5·10^{-3} mmol/l MCAA	8.014	19.86	5.619	25.08
		MCAA		Accompanying acid	
II	0.01 mmol/l MCAA + 7.5·10^{-3} mmol/l MBAA	7.493	25.07	5.624	25.01
	0.01 mmol/l MCAA + 7.5·10^{-3} mmol/l DCAA	7.489	25.11	5.776	22.98
	0.01 mmol/l MCAA + 7.5·10^{-3} mmol/l TCAA	7.497	25.13	5.847	22.04
	0.01 mmol/l MCAA + 7.5·10^{-3} mmol/dm^3 DBAA	7.501	24.99	5.854	21.95
		MBAA		Accompanying acid	
II	0.01 mmol/l MBAA + 7.5·10^{-3} mmol/dm^3 MCAA	7.493	25.07	5.635	24.87
	0.01 mmol/l MBAA + 7.5·10^{-3} mmol/l DCAA	7.507	24.93	5.779	22.94
	0.01 mmol/l MBAA + 7.5·10^{-3} mmol/l TCAA	7.493	25,07	6.013	21.83
	0.01 mmol/l MBAA + 7.5·10^{-3} mmol/l DBAA	7.489	25,11	6.009	21.88
		DCAA		Accompanying acid	
II	0.01 mmol/l DCAA + 7.5·10^{-3} mmol/l MBAA	7.713	22,87	5.619	25.07
	0.01 mmol/l DCAA + 7.5·10^{-3} mmol/l MCAA	7.703	22,97	5.615	25.13
	0.01 mmol/l DCAA + 7.5·10^{-3} mmol/l TCAA	7.666	23.34	5.803	22.63
	0.01 mmol/l DCAA + 7.5·10^{-3} mmol/l DBAA	7.659	23.41	5.806	22.59

study on the impact of chosen activators and inhibitors on the effectiveness of removal of HAA from water in the reactor with native biocatalyst are discussed in [16].

The analysis of the results obtained during the study makes it possible to conclude that three among five of the investigated acids act as competed inhibitors i.e. monochloroacetic and monobromoacetic acid for DCAA, TCAA, and DBAA, while dichloroactic acid regarding TCAA and DBAA. It is also shown that the immobilized catalyst is more resistant to inhibitor actions in comparison with its suspended form. Additionally, an increase in substrate concentration (TCAA and DBAA) results in much better limitation of inhibiting properties of MCAA, MBAA, and DCAA than in the case of using native enzymes.

For the purpose of determining the effect of selected ions on the removal rate of HAA mixture from water in the bioreactor with native enzymes, 100 cm^3 of aqueous solution of particular acids (0.01 mmol/l), the mixtures of five HAAs (MCAA, DCAA, TCAA, MBAA, and DBAA, each of 0.01 mmol/l), and 1 cm^3 of enzyme support were transferred to a 300 cm^3 flask with a ground glass joint, and salts of selected individual ions (chlorides and magnesium sulfhate ions) were added in the quantity appropriate for achieving their concentrations in samples at the level of 0.025, 0.25, 2.5, and 25.0 mmol/l, respectively. After checking pH of obtained solutions, they were thermostatted at 25°C for one hour. After that time, HAA concentrations were determined in the samples, and the enzymatic activity of the biocatalyst for each acid and the degree

Table 5
Composition of feed solutions and HAA concentrations at the third series of measurement tests on HAA biodegradation by ultrafiltration

		HAA concentration and biodegradation rates obtained after 1 h of the process			
		Basic acid		Accompanying acid	
Series no.	The feed composition and acids concentration	Concentration (mmol/l) × 10^3	B_d (%)	Concentration (mmol/l) × 10^3	B_d (%)
		DBAA		Accompanying acid	
III	0.01 mmol/l DBAA + $5 \cdot 10^{-3}$ mmol/l MBAA	7.614	23.86	3.734	25.32
	0.01 mmol/l DBAA + $5 \cdot 10^{-3}$ mmol/l DCAA	7.566	24.34	3.745	25.09
	0.01 mmol/l DBAA + $5 \cdot 10^{-3}$ mmol/l TCAA	7.479	25.21	3.741	25.19
	0.01 mmol/l DBAA + $5 \cdot 10^{-3}$ mmol/l MCAA	7.608	23.92	3.719	25.61
		TCAA		Accompanying acid	
III	0.01 mmol/l TCAA + $5 \cdot 10^{-3}$ mmol/l MBAA	7.621	23.79	3.807	23.85
	0.01 mmol/l TCAA + $5 \cdot 10^{-3}$ mmol/l DCAA	7.559	24.41	3.784	24.32
	0.01 mmol/l TCAA + $5 \cdot 10^{-3}$ mmol/l DBAA	7.509	24.91	3.748	25.03
	0.01 mmol/l TCAA + $5 \cdot 10^{-3}$ mmol/l MCAA	7.633	23.67	3.804	23.91
		MCAA		Accompanying acid	
III	0.01 mmol/l MCAA + $5 \cdot 10^{-3}$ mmol/l MBAA	7.478	25.22	3.734	25.32
	0.01 mmol/l MCAA + $5 \cdot 10^{-3}$ mmol/l DCAA	7.459	25.41	3.736	25.28
	0.01 mmol/l MCAA + $5 \cdot 10^{-3}$ mmol/l TCAA	7.477	25.23	3.744	25.19
	0.01 mmol/l MCAA + $5 \cdot 10^{-3}$ mmol/l DBAA	7.501	24.99	3.743	25.14
		MBAA		Accompanying acid	
III	0.01 mmol/l MBAA + $5 \cdot 10^{-3}$ mmol/l MCAA	7.506	24.94	3.751	24.97
	0.01 mmol/l MBAA + $5 \cdot 10^{-3}$ mmol/l DCAA	7.498	25.02	3.749	25.02
	0.01 mmol/l MBAA + $5 \cdot 10^{-3}$ mmol/l TCAA	7.478	25.22	3.743	25.14
	0.01 mmol/l MBAA + $5 \cdot 10^{-3}$ mmol/l DBAA	7.482	25.18	3.756	24.87
		DCAA		Accompanying acid	
III	0.01 mmol/l DCAA + $5 \cdot 10^{-3}$ mmol/l MBAA	7.624	23.76	3.750	25.00
	0.01 mmol/l DCAA + $5 \cdot 10^{-3}$ mmol/l MCAA	7.608	23.92	3.753	24.93
	0.01 mmol/l DCAA + $5 \cdot 10^{-3}$ mmol/l TCAA	7.557	24.43	3.751	24.97
	0.01 mmol/l DCAA + $5 \cdot 10^{-3}$ mmol/l DBAA	7.548	24.52	3.744	25.11

of its removal was calculated. Adding salts of appropriate ions to the reference water had no impact on changing pH of the feed solution. The obtained results were compared to the initial enzymatic activity of the biocatalyst, determined in the samples without any ion additives. No effect of Cl^-, SO_4^{2-}, Mg^{+2}, Zn^{+2}, Ca^{+2}, and Fe^{+3} ions was observed on the activity of native enzymes used for the tests. When Mn^{+2} ions were present, only a slight increase (by 3–4%) in enzymatic activity compared to the initial activity (without Mn^{+2}) was observed—Table 7. The increase in catalytic activity of the applied protein was not affected by the quantity of added Mn^{+2} ions. Also it had no impact whether an individual acid or the mixture of five HAAs underwent biodegradation.

The S-76-400 type reactor by Nuclepore company and PAN-17,5E membrane were used to determine the impact of selected ions on the rate of removing HAA mixture from water in the bioreactor with a flat, enzymatic ultrafiltration membrane. The individual acids (0.01 mmol/l) and the mixture of five HAAs with the added Cl^-, SO_4^{2-}, Mg^{+2}, Zn^{+2}, Mn^{+2}, Ca^{+2}, and Fe^{+3} ions in the quantity appropriate for achieving the concentration of 0.025, 0.25, 2.5, and 25.0 mmol/l were filtrated through that membrane for four hours. The transmembrane pressure was 0.1 MPa, the stirring intensity was 50 rpm, and the process temperature—25°C. Every hour the concentration of individual HAAs in the feed solution, permeate, and retentate was determined; the enzyme activity of the biocatalyst against each acid and its degree of removal were calculated. The obtained results were compared to the initial enzymatic activity of the biocatalyst, and the activity of PAN-17,5E membrane determined in

Table 6
Biodegradability degrees of HAAs in individual series of measurement tests on removing HAA from bioreactors with a flat, enzymatic membrane

Series no.	Biodegradation degree of HAA (%)				
	MCAA	MBAA	DCAA	TCAA	DBAA
I	25	25	22	20	20
II	25	25	23	22	22
III	25	25	24	24	24

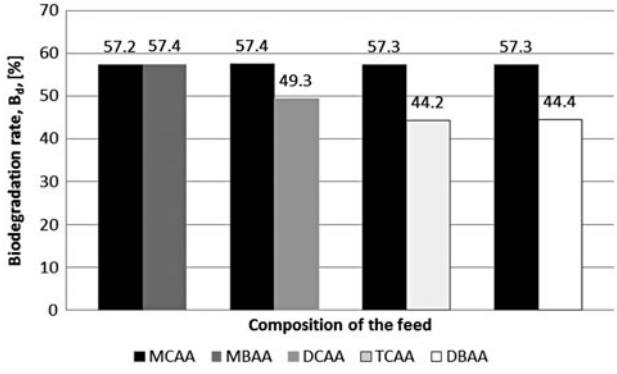

Fig. 3. The impact of the feed composition on DBAA removal rate (the first series of the study).

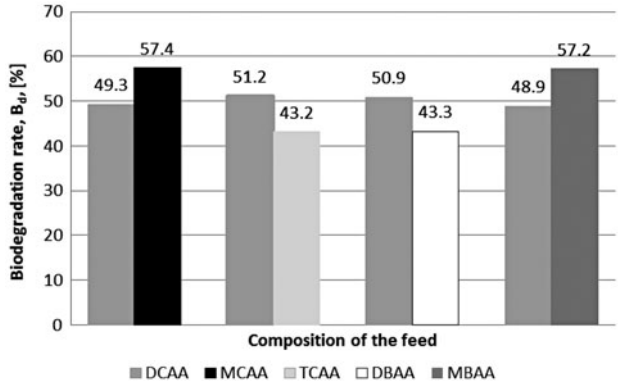

Fig. 4. The impact of the feed composition on DCAA removal rate (the first series of the study).

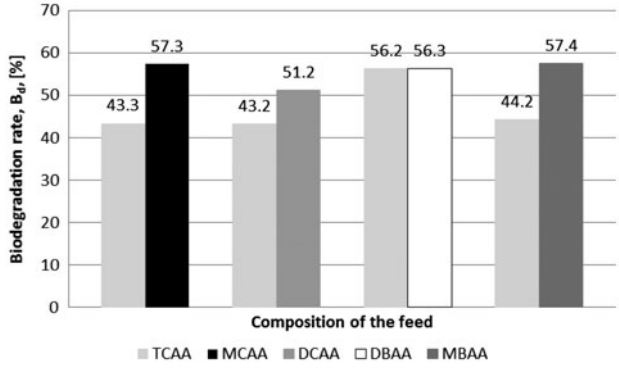

Fig. 5. The impact of the feed composition on TCAA removal rate (the first series of the study).

the samples without any ion additives. Similar to the case of removing the HAA mixture by means of native enzymes, no effect of ions on the activity of the immobilised biocatalyst, and thus on the process efficiency, was observed during the biodegradation of HAAs by ultrafiltration with enzymes immobilised on the polyacrylonitrile membrane.

Table 7
The comparison of enzymatic activity of the native biocatalyst determined at the presence and at the absence of Mn^{+2} ions

The amount of the enzyme (mg)	The initial activity of the enzyme with regard to the acid/the activity of the enzyme noted after the addition of manganese ions (mmol of acid/1 h) × 10^{-3}				
	(Mn^{+2}) = 0.025 mmol/l				
	MCAA	DCAA	TCAA	MBAA	DBAA
23,8	52,151/53,716	52,151/53,924	52,150/53,864	52,151/53,846	52,150/53,724
	(Mn^{+2}) = 0.25 mmol/l				
	52,151/53,724	52,151/53,841	52,150/53,921	52,151/53,732	52,150/53,817
	(Mn^{+2}) = 2.5 mmol/l				
	52,151/53,821	52,151/53,882	52,150/53,943	52,151/53,911	52,150/53,786
	(Mn^{+2}) = 25 mmol/l				
	52,151/53,785	52,151/53,729	52,150/53,879	52,151/53,794	52,150/53,977

5. Conclusions

The performed tests indicated that during the biodegradation of the mixture of five HAAs (MCAA, DCAA, TCAA, MBAA, and DBAA) in water, three of them behaved as competitive inhibitors by competing for the active site of the biocatalyst. Monochloroacetic acid and monobromoacetic acid are inhibitors for three other acids and their inhibition for DCAA is weaker than for TCAA and DBAA. Dichloroacetic acid turned out to be the inhibitor for TCAA and DBAA. Such a regularity was observed during HAA biodegradation by native enzymes (free state) as well as the enzymes immobilised on the ultrafiltration membranes.

No effect of Cl^-, SO_4^{2-}, Mg^{+2}, Zn^{+2}, Ca^{+2}, and Fe^{+3} ions was observed on the activity of applied biocatalysts, and, consequently, on the process efficiency. Only a slight increase (by ca. 3–4%) in catalytic activity of native enzymes was observed in the presence of Mn^{+2}. For immobilised enzymes, no effect of added ions on their catalytic activity was observed.

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