



Removal of organic matter through full-scale drinking water biofilters

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ABSTRACT

The research was carried out in a full-scale water treatment plant (WTP) in Poland to which water is supplied from ground and infiltration water intakes. The WTP has recently launched the second stage of the water treatment process based on the combination of ozonation and biologically activated carbon (BAC) filtration. The samples of water and the filter bed were collected once a month. Physical and chemical analyses were carried out, as well as the identification of bacterial species by way of biochemical diagnostics. The investigation proved that the examined carbon filter was biologically active. A correlation between the decrease in the dissolved oxygen concentration and the efficiency of carbon compounds removal was found. It was concluded that the total number of psychrophilic bacteria had impact on the biofiltration efficiency. The microbiological activity of treated water was rising during filtration, while the microbiological activity of the filter bed was relatively stable. Two microorganisms, *Sphingomonas paucimobilis* and *Pseudomonas fluorescens*, belonging to bacterial species typically inhabiting BAC filters, were identified. The research showed the absence of any members of the Enterobacteriaceae family in water or the filter bed. Studies have confirmed that FDA test is fast, simple and inexpensive, so it can be an effective tool in the routine control of biodegradation of organic matter in biofilter beds.

Keywords: Biological activated carbon; Biofiltration; Drinking water; FDA test; Microbiological activity; Organic matter

1. Introduction

The tightening of the requirements for the quality of drinking water often necessitates the upgrading of the existing water treatment systems. Sorption on activated carbon becomes an integral component of modern WTPs. Granular activated carbon (GAC) filtration has become a main barrier in surface-water treatment for the removal of

organic contaminants. GAC filtration removes from water natural organic matter, including humic and fulvic acids and biodegradable compounds. Biologically active carbon filters are multifunctional. In addition to the basic process which is the adsorption, biochemical processes take place with the participation of microorganisms inhabiting filters [1–6]. Thanks to a suitable porous structure and highly developed surface, activated carbon adsorbs both organic and inorganic compounds from water, including

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some heavy metals [7]. It is necessary to pay attention to appropriate preparation of active coal already at the stage of its production [8]. An important element of an effective biochemical process is to be preceded by water ozonation, during which some of the compounds resistant to biochemical degradation are transformed into biodegradable forms. Currently, great importance is attached particularly to the removal of ubiquitous biodegradable organic matter fraction present in aquatic ecosystems [9–11]. This fraction includes a heterogeneous mixture of organic compounds with different physicochemical properties, including proteins, amino acids, lipids, polysaccharides and biopolymers [12–15]. The presence of a biodegradable fraction of organic matter in the water can cause the secondary development of microorganisms in the water supply network and thus worsen the organoleptic characteristics of water directed to the consumer [16–18]. Removal of this fraction of material is possible with a biofiltration process [19–29]. The combination of ozonation and BAC is an effective treatment process for water containing organic matters, which have been proved by numerous researches. The effect of microorganism on the activated carbon is effective to eliminate ammonia nitrogen and enhance the filtration process [30–34].

The aim of the study was to assess the impact of the microbiological activity on the efficiency of organic compounds removal from water treated on recently launched carbon filters of analyzed WTP. The WTP has recently launched the second stage of the water treatment process based on the combination of ozonation and biologically activated carbon (BAC) filtration. In Europe and in the world, there are many WTPs, which use biologically active carbon filters. These installations were turned on many years ago. During this time, there were no such analytical capabilities as at the present time. The modernization of the WTP created the possibility of collecting water samples into the bed profiles appeared, which is unprecedented in the technical scale. Therefore, the presented research results are exceptional. In addition to traditional culturing methods, the metabolic activity assay (FDA method) was used to evaluate the microbiological activity of the filter bed and of the water collected in the filter's bed cross-section. This test allows to estimate the efficiency of BAC filters. The test is fast, simple and inexpensive, so it can be an effective tool in the routine control of biodegradation of organic matter in biofilter beds.

2. Materials and methods

2.1. Object of research

The WTP, after a thorough modernization completed in 2015, has a maximal water production capacity of 150,000 m³/d. There are three technological lines with a maximum capacity of 50,000 m³/d each. The WTP is supplied with underground and infiltration waters with a different chemical composition. The groundwater is characterized by a high general hardness, high concentration of iron and manganese compounds and high concentration of substances in the form of dissolved carbonate, chloride and sulfate ions. The groundwater is sourced from geological

layers formed from dead plant remains, transformed by the humification process, whereby the captured water contains specific dissolved organic matter, while the surface water contains a significant amount of organic compounds, due to the quality of the river water. During the 2012–2015 study, which focused on the evaluation of the quality of raw water delivered to the WTP, it was found that the organic matter concentration, measured by TOC, was 5.3 g C/m³ on average, ranging from 3.4 g C/m³ up to a record high of 12.0 g C/m³ measured in summer 2013, when intense floods occurred in the river basin.

The raw water treated at the analyzed WTP during the research period was characterized by iron content ranging from 0.64 to 6.4 mg Fe/L, the concentration of manganese from 0.46 to 0.79 mg Mn/L, elevated turbidity from 3.5 to 19.0 NTU and water colour from 7.5 to 15.0 mg Pt/L. The content of organic compounds was quite high, as evident from the following parameters: TOC from 4.4 to 6.4 mg C/L and UV absorbance at 254 nm ranged from 5.8 to 16 m⁻¹. Selected parameters of the raw water are presented in Table 1.

The raw water is treated in a technological system based on aeration, first and second stage of rapid filtration, ozonation, and sodium hypochlorite and chlorine dioxide disinfection. The scheme of the WTP is presented in Fig. 1.

Aeration and the concurrent degassing of water take place in an open system of 30 aeration cascades. Some of the iron compounds that have been oxidized are precipitated in the form of flocs falling down to the bottom of the reaction chambers under the cascades. The chambers can also be used for pre-oxidation of iron and manganese compounds. Subsequently, water flows into the second-stage reaction chambers, where the active carbon may be dosed in the event of a sudden deterioration of the raw water quality. Thereafter, water is directed to the first stage filters. Each process line has 10 filtration chambers filled with a fluid double-layer quartz-anthracite filter bed, wherein the processes of iron and manganese removal take place.

The second stage of water treatment is based on the integrated processes of filtration and ozonation through a BAC filter bed. Its main purpose is to remove dissolved organic matter from water and thereby lower the dose of disinfectants, so that the water has a more natural flavor and odor. Ozone is produced from technical oxygen and is dosed into the water in a gaseous form. It oxidizes organic compounds, part of which becomes easily eliminated by microorganisms inhabiting the BAC filter. This is followed by the degassing and pumping of water to carbon filters building. There are eight filter chambers filled with granular activated carbon per each process line, with a bed height of 2 m each. Each of the 24 filter chambers has internal dimensions of 6.9 × 5.7 m, so the surface of one carbon filter is 39.33 m² and the total filtration surface of the entire WTP is 943.92 m². The filtration chambers are filled with WG-12 activated carbon (manufacturer: Gryfskand Sp. z. o.o., Hajnówka, Poland) made of special, low-ash coal, connected by a binder and activated by water vapor (iodine quantity 1,100 mg/g, methylene blue adsorption 30 g/100 g; total surface area BET 1,100 m²/g; particle size 1.5–0.75 mm). The maximum hydraulic load of filtration equals 9.0 m³/m²h. After 24 d of operation or when the resistance rises to 1.5 m H₂O, the filter is backwashed with water and air flow providing depressurization of the

Table 1
Selected raw water parameters

Parameter	Number of analyses	Raw water		
		Min.	Max.	Average
Color, mg Pt/L	50	7.5	15.0	10.95
Turbidity, NTU	50	3.5	19.0	8.67
pH	50	7.2	7.6	7.37
Iron, mg Fe/L	50	0.64	6.4	1.56
Manganese, mg Mn/L	46	0.46	0.79	0.61
Total organic carbon, mg C/L	50	4.4	6.4	4.97
Absorbance at 254 nm, cm ⁻¹	50	58	160	127.4

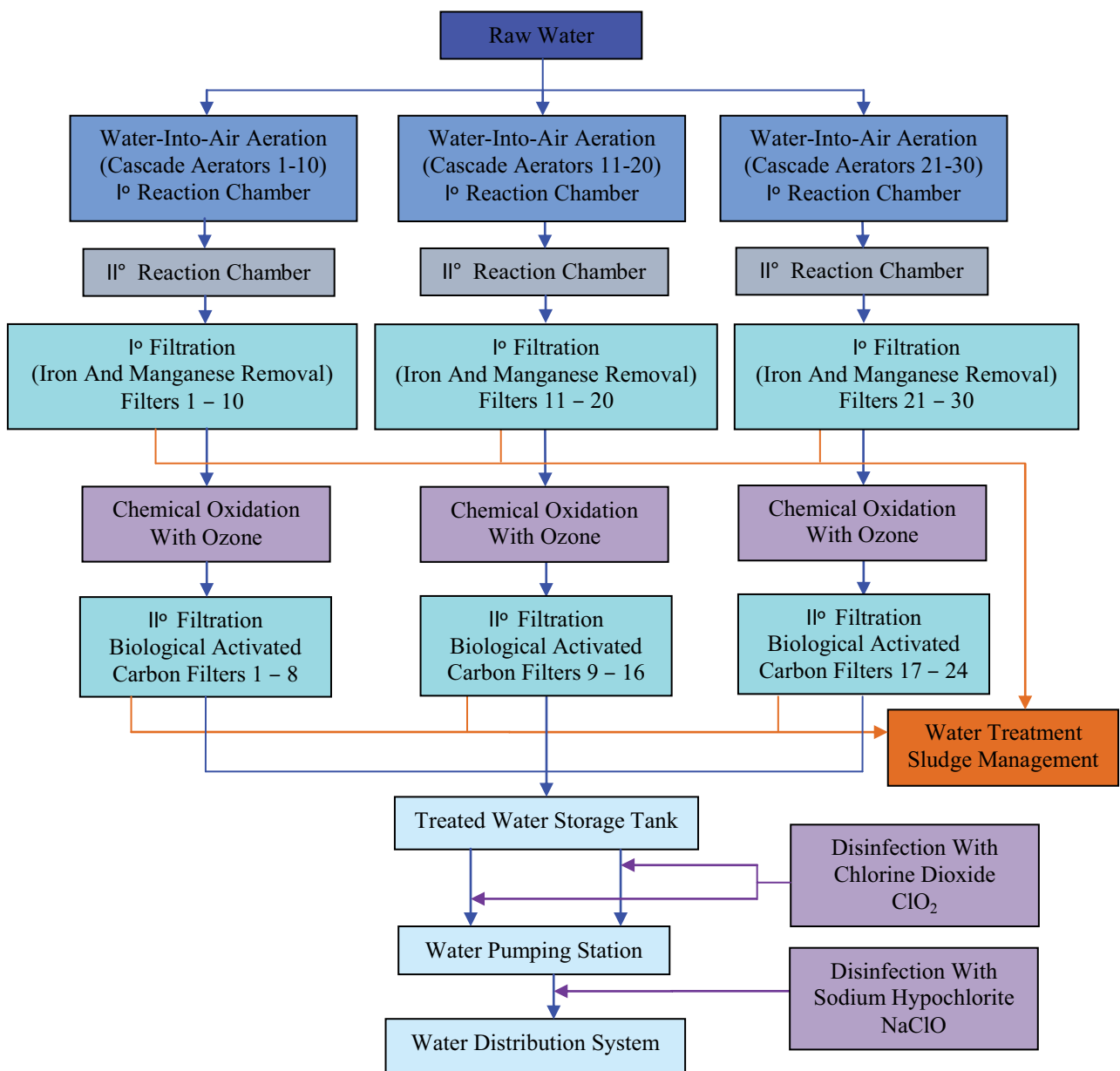


Fig. 1. Scheme of the water treatment plant.

filter bed by 35%–50%. The backwashing water is discharged via troughs with an inclined bottom. Neither the anthracite-quartz beds nor the activated carbon filter beds have any supporting layer. It has been possible to omit it thanks to the use of a modern panel drainage system with stainless steel caps. The coal filters operated in the WTP were filled with fresh activated carbon WG–12 in December 2014 and started operating in January 2015. The study of water quality filtered through the filter bed showed that the efficiency of organic matter removal was high during the initial phase of the carbon filters operation, which indicated the existence of the process of chemisorption. With time, the TOC removal efficiency gradually decreased until it stabilized at 10%–20%. The analysis of changes in the TOC concentration and UV absorbance indicates that the biosorption phase started at the end of March 2015.

Treated water during the research period was characterized by low iron concentration from 0.000 to 0.021 mg Fe/L, manganese concentration from 0.000 to 0.014 mg Mn/L, turbidity from 0.13 to 0.58 NTU and water color from 0 to 5 mg Pt/L. The content of organic compounds (TOC) ranged from 3.5 to 5.2 mg C/L and UV absorbance at 254 nm from 5.7 to 9.7 m⁻¹. Selected parameters of the treated water are presented in Table 2. Treated water fulfilled the requirements for the quality of water intended for human consumption [35,36].

The view of the WTP is presented in Fig. 2.

2.2. Methods

During the sampling procedure, water passed through the filter, but due to high quality requirements for water delivered to customers, at the time of interference with the filter due to sampling the whole filtrate was directed to the sewage system.

The water was collected at inflow into the filter (after ozonation), across the filter bed at the height of 1.20, 1.55, 1.90 m and at the filter outflow. The analyzed filter was in operation since January 2015 and was adjusted to water sampling from the filter cross-section (Fig. 3).

The water samples were collected into sterile 300 mL bottles. Immediately after collection, the following water analysis was determined in accordance with Standards Methods: alkalinity, dissolved oxygen concentration, pH,

temperature and conductivity. After transporting the samples to the laboratory, COD with KMnO₄, TOC, the total number of psychrophilic bacteria and the microbial activity (FDA test) were additionally determined. Research has focused on psychrophilic microorganisms, which are bacteria or archaea having an optimal growth temperature at about 15°C or lower, a maximal growth temperature at about 20°C and a minimal temperature for growth at 0°C or lower. The temperature of the water to be purified on the analyzed WTP was within the mentioned range. The number of psychrophilic bacteria was determined after their deep inoculation and growth on enriched agar (HPC method) and the metabolic activity of biomass was measured by the FDA method [37,38].

The HPC method is a basic and relatively simple method of determining the number of heterotrophic microorganisms. It can be carried out in microbiological laboratories with a basic equipment. After inoculating bacteriological samples under specific conditions (e.g., thermal or oxygen) on the appropriate growth medium, the number of different groups of bacteria can be determined. In practice, when examining the microbial activity of bed-settling bacteria, the most commonly determined factor is the total number of microorganisms incubated at 22°C for 72 h or saprotrophic psychrophilic bacteria. The precise methodology of the determination of psychrophilic bacteria in drinking water is described in Polish standard no. PN-EN ISO 6222:2004 [38].

The carbon filter bed samples were taken from the upper surface layer at five locations of the carbon filter no. 5, along the operating platform. The samples of the carbon filter bed were collected with a sterile steel pipe to sterile 100 mL bacteriological containers. The filter layout and the sampling points are shown in Figs. 4 and 5.

To obtain the number of psychrophilic bacteria in the filter bed, about 2 g of the carbon filter bed sample was placed in a 250 mL flask containing 100 mL of sterile water. Then the flask was placed in a shaker for 30 min. After shaking, the liquid from above the grains was collected with a sterile pipette and poured onto sterile Petri dishes covered with about 10 mL of medium agar (nutrient agar). Then the plates were incubated for 72 h at 20°C ± 2°C. After the incubation period, the bacterial colonies were counted and prepared for further microbiological diagnostics. All results were calculated to the colony forming units in 1 mL of the sample (CFU/1 mL).

Table 2
Selected treated water parameters

Parameter	Number of analyses	Treated water			Regulation of the Minister of Health [36]
		Min.	Max.	Average	
Color, mg Pt/L	201	b.d.	5	1.41	<15 mg Pt/L
Turbidity, NTU	201	0.13	0.58	0.28	<1.0
pH	201	7.1	7.8	7.44	6.5–9.5
Iron, mg Fe/L	51	b.d.	0.021	0.009	0.2
Manganese, mg Mn/L	47	b.d.	0.014	0.005	0.05
Total organic carbon, mg C/L	51	3.5	5.2	4.22	Without abnormal changes
Absorbance at 254 nm, cm ⁻¹	51	5.7	9.7	73.3	–

b.d. – below detection.



Fig. 2. View of the water treatment plant with a maximum water production capacity of 150,000 m³/d (<https://www.ptbnickel.pl>).



Fig. 3. Installation for collecting water from the filter bed cross-section.

Biochemical diagnostics (BioMerieux's Vitek 2 Compact is installed at the Greater Poland Cancer, Microbiology Laboratory in Poznan, Poland) was used to identify the bacterial species. For the study, two sets of environmental monitoring samples were taken. Each of the samples was cultured on chromID™ CPS and Mueller Hinton agar (bioMerieux). All plates were inoculated with a matched set of

1.0 mL pipettes by applying 0.2 mL portions of the sample evenly over the surface. Set one was designed to detect psychrophilic and psychrotolerant microorganisms. With the second set, similar samples were taken and incubated according to the mesophilic incubation regime. The incubation regime:

- Psychrophilic test: 2°C–8°C, minimum of 7 d.
- Mesophilic test: 30°C–35°C, minimum 3 d, followed by: 20°C–25°C, minimum 6 d.

After incubation period colonies on the plates could be counted and the concentration of bacteria in the original culture was calculated. Depending on the bacterial species and the culture conditions, colonies can exhibit a great diversity of forms. Not only are pigment differences seen but also size, edge, pattern, opacity, and shine. In order to multiply the bacteria, a single agar-air colony was picked from a culture plate with an inoculation needle and transferred into the Mueller–Hinton agar. Isolates detected from the psychrophilic and mesophilic study were identified using a phenotypic Vitek identification system (bioMerieux, Marcy l'Etoile, France), which is a tool for identification of the most isolated strains to the genus level and many others to species level.

The measurement of the fluorescein diacetate (FDA) hydrolysis rate (fluorescein luminescence intensity) was performed using the PerkinElmer Instruments LS55 luminescence spectrometer (Located at the Institute of Environmental Engineering and Building Installations of Poznan University of Technology, Poland), at the excitation wavelength of 433 nm and the emission wavelength of 525 nm. The spectrometer was connected with a computer equipped with the appropriate FL WinLab software. To determine the microbial activity (esterase activity EA), 3 mL of the sample (water or liquid after shaking the filter bed) were pipetted and poured into a sterile cuvette than, 120 µL of fluorescein diacetate in acetone (FDA) was added just before measurement. Each sample was mixed

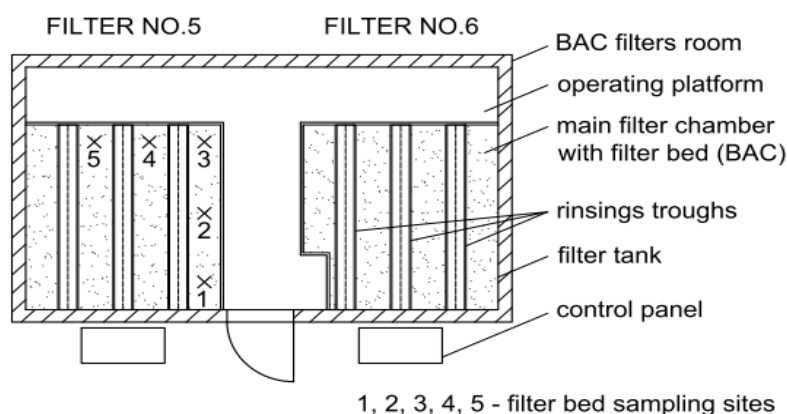


Fig. 4. Layout of the filter room and sampling sites.



Fig. 5. Filter chamber.

and placed in a fluorimeter LS 55 Luminescence Spectrometer from Perkin for 10 min. FL WinLab software was used to read the value of the microbial activity (EA) and subsequently the increase in EA over time was plotted. After the adaptation time, in all samples, FDA hydrolysis occurred. The metabolic activity of microorganisms was read from the slope of the straight line representing the relationship of the luminescence intensity of the resulting fluorescein and time, for a period of 5–10 min. The following method of determining the EA value was used: in the case of water samples, the test result was the EA calculated on the basis of the slope of the fluorescence intensity interval, from the time the graph was broken until the end of the test, while in the case of the samples of liquid from above the filter bed, the entire time interval of 600 s (10 min) was used to calculate the EA. The value was expressed in relative units per second (r.u./s).

3. Results and discussion

The biological activity of the filter bed is related to the presence of microorganisms in the water and consists of forming of the biological layer on the surface of the filter

grain. This process lasts from several to several weeks and depends on many factors such as: water temperature, type and concentration of organic compounds, oxygen concentration and type and granulation of the filter material. The pH of the incoming water, the type of pollutants and the concentration of toxic substances are also important [22,39–51].

For microorganisms colonizing biologically active filter beds, a biologically active source of carbon and energy is biodegradable dissolved organic carbon. Removal of organic matter from water is a result of oxidation in the respiratory processes of microorganisms and the increase of their biomass. Decrease in oxygen concentration and following increase in carbon dioxide concentration in the treated water indicates the development of microorganisms in the filter bed [3–5,39–43]. Due to the high effectiveness of dissolved organic matter removal by biologically active carbon filters, the treated water requires much less disinfectants. In such water, the likelihood of harmful by-products formation or bacterial regrowth in the distribution network is much lower. Therefore, it is extremely important to continuously control the biofiltration process, especially the metabolic activity of the biomass, which enables proper

control of biofilters' work and eliminates or limits the phenomena disturbing their work. The microbial growth in the filter bed is evidenced by a decrease in the oxygen concentration, combined with the increase in carbon dioxide concentration in the treated water [3,4,39,44]. Bacteria colonizing the filter beds are mainly psychrophilic, both auto- and heterotrophic, but only the heterotrophic bacteria are responsible for the decomposition of organic compounds adsorbed on the surface of activated carbon grains. Among the bacteria that predominate are the genus *Pseudomonas* sp. (*Maltophilia*, *Pseudomonas cepacia*, *Pseudomonas acidoverans*) and *Acinetobacter* sp., *Flavobacterium* and *Bacillus* sp. [45]. The colonization of the filter by microorganisms forms vertical stratification, as a result of the difference in the oxygen concentration and nutrient content at different depths of the filter bed [46,47]. The biological activity of the filter bed is related to the presence of microorganisms in the water and consists of forming of the biological layer on the surface of the filter grain. This process depends on many factors such as water temperature, type and concentration of organic compounds, oxygen concentration and type and granulation of the filter material. The pH of the incoming water, the type of pollutants and the concentration of toxic substances are also important [22,39,40,45,48]. The rate of biological layer development also depends on the amount and type of bacteria and other microbes present in the

water. The growth of microorganisms on the surface of the filter bed should be controlled not only to prevent colmatation of the bed but also to prevent the risk of occurrence of pathogenic microorganisms. The quality of the effluent water from biologically activated sorption filters depends on the type of activated carbon used, its adsorption properties and the intensity of the biochemical processes taking place in the activated carbon bed. Both qualitative and quantitative composition of the microbial populations of the activated carbon deposit are very important [22,40,49].

The water flowing through carbon filter no. 5 was filtered at a rate ranging from 2.5 to 3.2 m/h. Due to the number of days since the last backwash of the filter bed, the analyzed filter had the highest water flow resistance during sampling in March, and the lowest in May. The basic parameters of the filter operation are summarized in Table 3. Table 4 shows the quality of water flowing into carbon filter no. 5.

The pH of the supplied water remained stable in the range of 7.30 to 7.53 and the inlet temperature varied from 9.40°C to 13.50°C. In all months, as a result of biofiltration, the pH of the filtered water decreased, but the changes were insignificant and amounted to a maximum of 0.23 and a minimum of 0.03. Due to the introduction of the ozonation process before BAC filtration, the concentration of dissolved oxygen in the water supplied to the filter was high and ranged from 10.52 to 14.43 mg O₂/L (Fig. 6). In April,

Table 3
Parameters of BAC filter no. 5 during sampling

Parameters of filter no. 5	Date of sampling					
	November	January	February	March	April	May
Volumetric flow rate at the time of collecting samples (m ³ /h)	126	121	74	47	N/A	33
WTP's daily water production (m ³ /d)	N/A	68,541	64,980	56,660	72,356	58,042
Average hourly flow (m ³ /h)	–	119	113	98	126	101
Filter surface area (m ²)	39.33	39.33	39.33	39.33	39.33	39.33
Average filtration velocity (m/h)	–	3.0	2.9	2.5	3.2	2.6
Height of the filter bed (m)	2	2	2	2	2	2
Average contact time (min)	–	40	42	48	38	47
Filter operating time since last backwash (d)	9	18	14	24	11	7

Table 4
Quality of water supplied to BAC filter no. 5 during sampling

Quality of water supplied to filter no. 5	Date of sampling						Average value	Standard deviation
	November	January	February	March	April	May		
pH	7.30	7.41	7.52	7.40	7.53	7.48	7.44	0.087
Temperature (°C)	13.50	10.30	10.20	9.40	9.40	9.60	10.40	1.568
Dissolved oxygen concentration (mg O ₂ /L)	13.21	12.28	10.92	10.66	13.75	13.44	12.38	1.326
Conductivity (μS/cm)	546	741	755	850	840	582	719	128.281
Alkalinity (mval/L)	3.63	5.56	3.93	4.10	4.00	3.97	4.20	0.685
Alkalinity (mg CaCO ₃ /L)	181.25	277.75	196.50	204.75	200.00	198.50	209.79	34.230
COD (KMnO ₄) (mg O ₂ /L)	–	–	3.18	3.63	4.37	5.87	4.26	1.176
TOC (mg C/L)	3.80	3.80	3.70	3.80	3.90	4.00	3.83	0.103

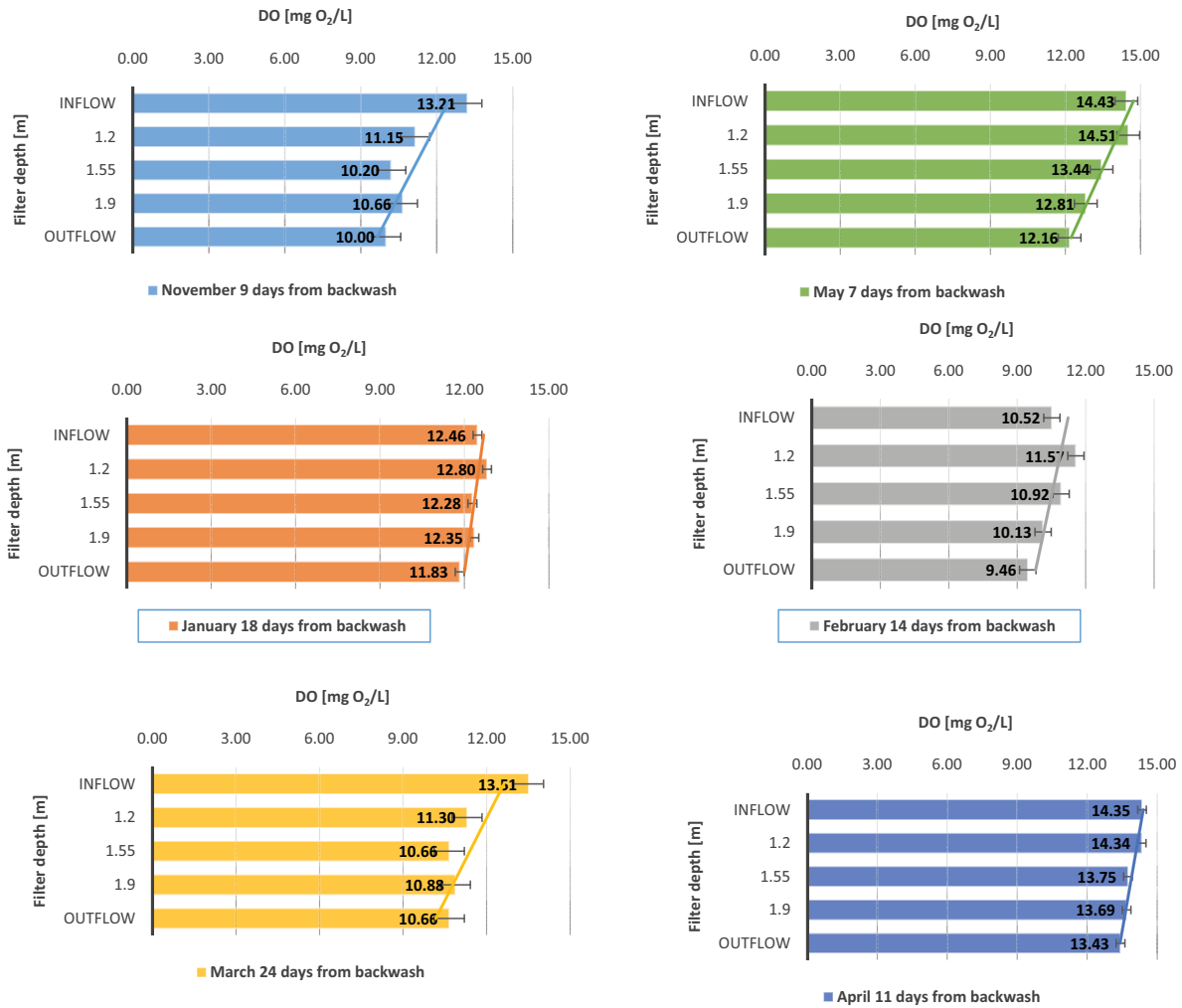


Fig. 6. Change in dissolved oxygen concentration in water during filtration through filter no. 5.

the reduction of dissolved oxygen in water as a result of the water filtration was the lowest of all analyzed months and amounted to 0.92 mg O₂/L, while in other cases it ranged from 1.06 to 2.85 mg O₂/L.

The TOC concentration in the inflow water was relatively stable and ranged from 3.7 to 4.0 mg C/L. The higher values occurred in warmer months. Except for water samples in May, the water past the first treatment stage showed COD with KMnO₄ values not exceeding the limit of 5 mg O₂/L (Fig. 7). The maximum reduction of COD was 2 mg O₂/L in May and the minimum was 0.74 mg O₂/L in February, hence the efficiency of COD reduction amounted to 23% – 34%. It was evident that the COD with KMnO₄ of both the inflow and outflow water increased from month to month, which may be related to the increase in the temperature of air and of the supplied water between February and May. It is believed that more organic compounds occur in water during warm periods vs. the winter months.

An indirect method for determining biological activity in BAF is the Eberhardt, Madsen and Sontheimer (EMS) test based on the determination of the coefficient S:

$$S = \frac{\Delta[\text{COD}]}{\Delta[\text{DO}]} \tag{1}$$

where Δ[COD] (mg O₂/L) – the reduction of the chemical oxygen demand of water; Δ[DO] (mg O₂/L) – the loss of dissolved oxygen in water.

The test EMS is useful in determining the relationship between the adsorption and the biodegradation process in the BAF bed, assuming that organic compounds are removed both by way of sorption and biodegradation, and oxygen is consumed by aerobic microorganisms to oxidize carbon. If S = 1, both adsorption and biodegradation proceed with the same intensity in the filter bed. If S > 1, adsorption predominates, and if S < 1, biodegradation is predominant. When S and Δ[COD] equal 0, the sorption and biodegradation processes are stopped. If Δ[COD] > 0 and Δ[O₂] = 0, sorption is present and biodegradation does not occur. In turn, when both Δ[COD] and Δ[O₂] are equal 0, neither of the processes takes place [24].

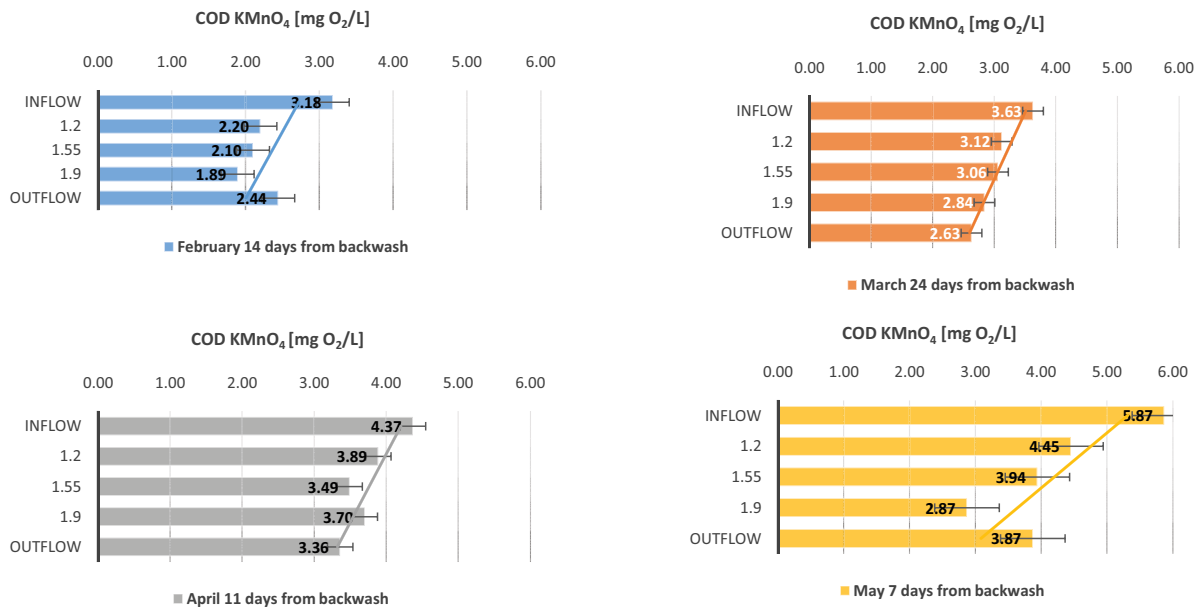


Fig. 7. COD KMnO_4 reduction during filtration through filter no. 5.

A slight decrease of dissolved oxygen concentration during BAC filtration resulted in an increase in the EMS indicator, which on April was the highest of the whole study period and amounted to 1.1, indicating that the sorption process prevailed over biodegradation (Fig. 6). As a result, in April, the TOC reduction was the lowest of all analyzed months and equalled 8%, while the highest value of 25% was recorded in May. The average value of the TOC reduction was 16% (Fig. 8).

A comparison of Figs. 6–8 shows that the high EMS indicator does not correlate with the reduced efficiency of the COD reduction, but results from a minor decrease in the dissolved oxygen concentration. Throughout the study period, the number of psychrophilic bacteria varied considerably (Fig. 9). The lowest bacteria count was recorded in January and April, while the highest growth occurred in March. On the day of the sample collection in March, the filter was backwashed – it was the 24th day of the filter operation. The backwash took place after sampling, which means that the water used for the bacteria subculturing had theoretically the highest possible content of biomass.

Worth noting is the fact that on the days of sampling where the number of psychrophilic bacteria was higher (February, March, May), the reduction of dissolved oxygen concentration was also higher (Fig. 6). The high reduction of dissolved oxygen concentration equal to $2.85 \text{ mg O}_2/\text{L}$ coincided with the highest bacterial count, proving that the filter was inhabited by microorganisms consuming oxygen in order to carry out life processes. In all assays, the lowest numbers of bacteria were cultured from samples of water supplied to the filter after ozonation, due to the bactericidal action of ozone. In almost all months, most bacteria were cultured from the sample taken at the depth of 1.20 m. With the increasing depth, the number of psychrophilic bacteria decreased. Some psychrophilic bacteria are undemanding in the presence of nutrients and environmental conditions. They can develop in both – aerobic and

anaerobic – conditions, therefore their presence at various depths of the filter bed can be associated with different physical–chemical parameters of the filter bed.

Although the total number of psychrophilic bacteria in water samples collected on April was lower than on May, the filter showed higher microbial activity in April. In April, during sampling, the BAC filter was 11 d in operation since the backwash, while in May the samples were taken 7 d after the backwash. It can be concluded that the operating time of the filter bed was related to an increased microbial activity (Fig. 10). Both in April and May, microbial activity in water tended to increase with the increasing depth of sampling (Fig. 10).

The relatively high microbiological activity in the water after ozonation in April is surprising, as ideally ozonation should stop the life processes of bacteria.

Although the microbial activity in water was higher on April than on May, the EMS indicator was high on that day ($\text{EMS} = 1.1$) and indicated that the adsorption process prevailed over biodegradation. The result was a lower reduction of both TOC and COD in April than in May. The COD reduction in May was the highest of all recorded during the period under review (34%), while in the period from February to April it ranged from 23% to 28%. In spite of the high EMS indicator, the decrease in the oxidation value in April (23%) was similar to the remaining months. The TOC showed a different pattern. In April, the TOC decrease was clearly lower (8%) than in the remaining months (11%–25%). The highest TOC removal efficiencies were recorded in May, which can be attributed to a more intense microbial growth during the periods of higher outdoor temperatures.

The study of microbiological activity in water suggests that even lower biomass activity levels ensure effective reduction of organic compounds determined on the basis of the TOC and COD in the treated water. It was found that the microbiological activity of the BAC filter bed at the level

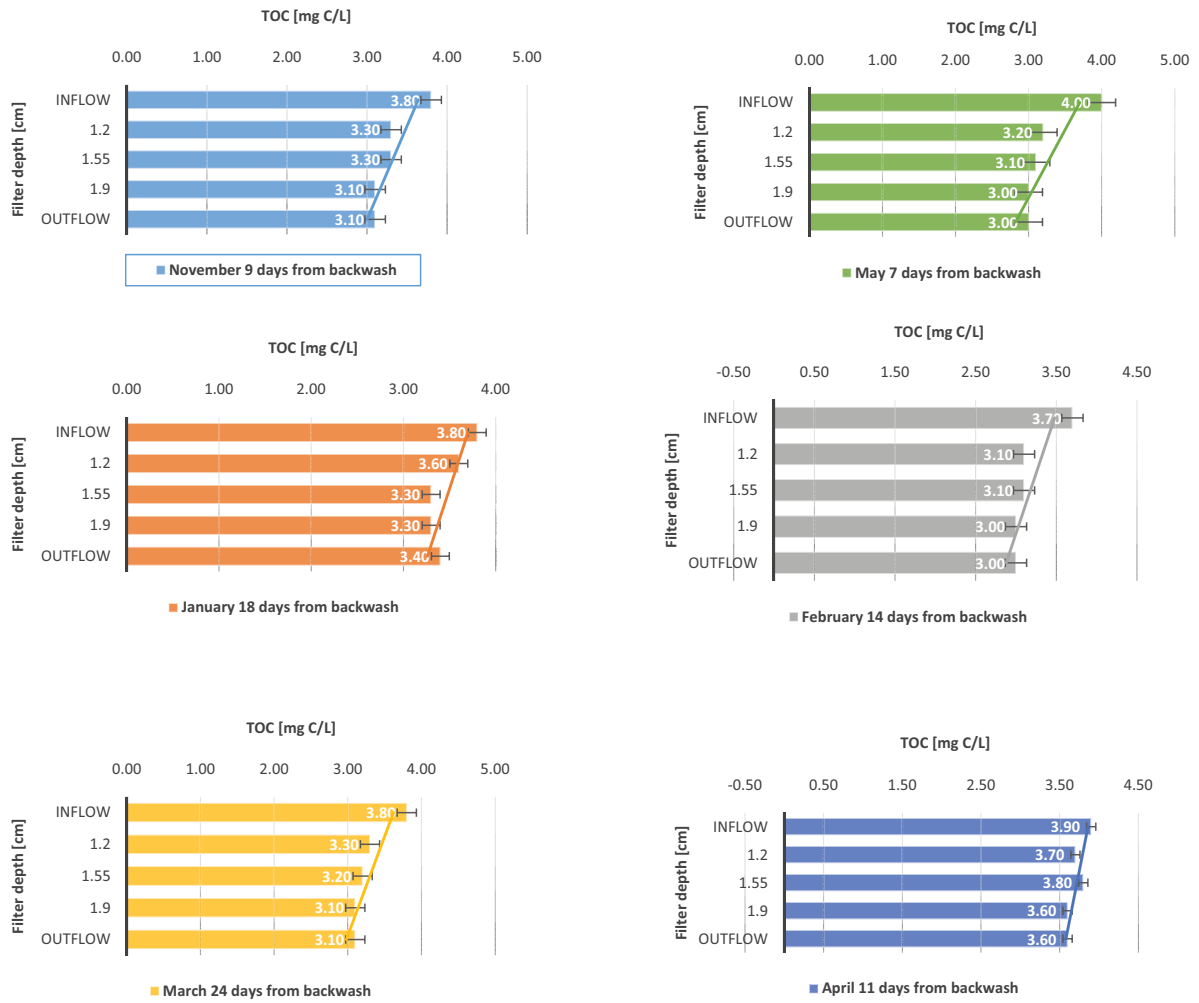


Fig. 8. TOC reduction during filtration through filter no. 5.

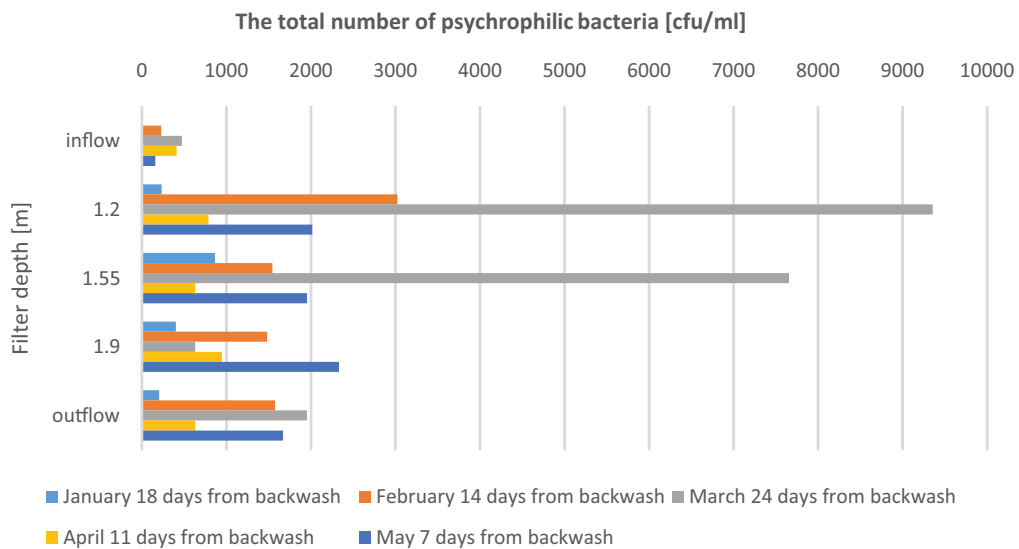


Fig. 9. Number of psychrophilic bacteria across the filter bed in water samples.

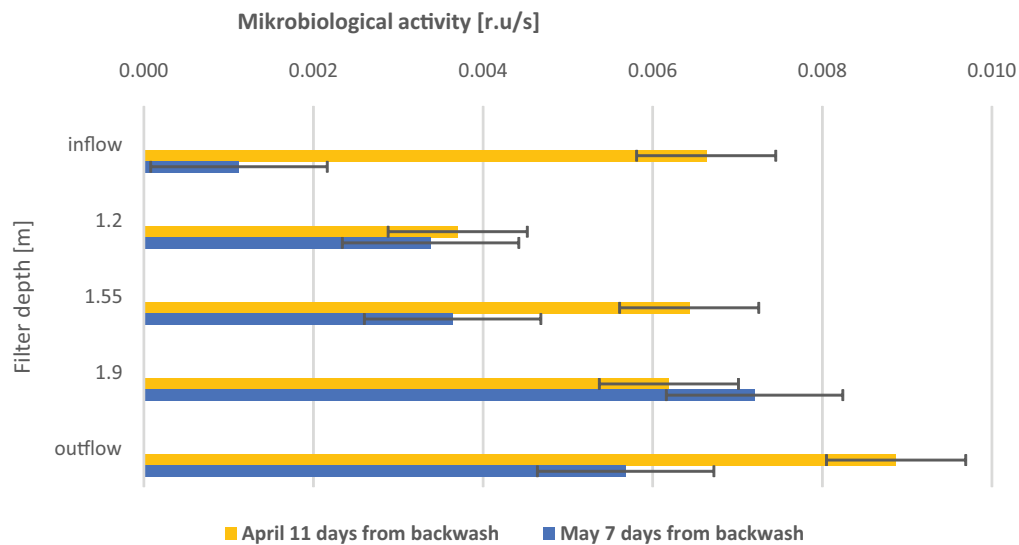


Fig. 10. Microbial activity in water samples across the filter bed.

of 0.3512–0.665 r.u./s was sufficient to satisfactorily remove TOC and COD from water. The efficiency of water biofiltration is significantly impacted not only by the biomass activity but also by the overall number of bacteria responsible for biodegradation. The increased microbial activity in April did not compensate for the scarcity of bacteria, which resulted in the deterioration of the efficiency of organic pollutants removal from water.

The identification of bacterial colonies cultured in Petri dishes showed the presence of two species of bacteria. *Sphingomonas paucimobilis* was found in the surface layer of filter no. 5, and *Pseudomonas fluorescens* was detected in the slurry from the filter bed and in samples collected from the water above the filter bed. The *Pseudomonas* sp. bacteria are Gram-negative microorganisms living in water and soil all over the world. They are able to decompose a wide range of organic substances, including a significant proportion of aromatic and aliphatic hydrocarbons, and therefore play a particularly important role in biofiltration of water [3,42]. *Sphingomonas paucimobilis* belong to the group of aerobic Gram-negative bacilli with a writhe and yellow pigment, which do not ferment glucose. They are capable of surviving in the environment with low nutritional content and can be detected even in the ultrapure water for industrial and medical purposes. Due to the fact that coal is their main source of energy, they have the ability to biodegrade many organic compounds [52]. Neither water nor the filter bed tests identified any Enterobacteriaceae bacteria, indicating that no coli or fecal coliform bacteria were present in the analyzed filter.

4. Conclusions

The research conducted on the technological scale has shown that during the study period carbon filter no. 5 was biologically active. It was confirmed by the decrease in the concentration of total organic carbon and the microbial activity. The microbiological activity of the bed was confirmed by the value of the EMS indicator, which was lower than one, and the bacteriological tests of the water and the filter bed.

The esterase activity assay with fluorescein diacetate (FDA) allowed for the assessment of the activity of the biomass in the filter bed. It has been found that the microbial activity of the filter bed ranging from 0.3512 to 0.6568 r.u./s is sufficient to remove organic matter from water, primarily in the process of biodegradation.

Water filtration through a BAC deposit lowered the organic content in water. The studies have shown a reduction in the content of organic compounds expressed by changes in oxygen consumption: COD (on average by 27%) and TOC (on average by 16%). The reduction of organic content at different heights of the filter bed correlated with the number of psychrophilic bacteria growing in the bed.

The backwash process influenced the quantity of bacteria present in the filter bed. Prior to the filter backwash, the overall number of bacteria was high and decreased afterwards.

The bacteria identification procedure showed that two bacterial strains were present in BAC filter no. 5: *Sphingomonas paucimobilis* and *Pseudomonas fluorescens*. These bacteria are microorganisms typically colonizing the beds of BAC filters. They are capable of removing organic compounds from water, which undoubtedly contributes to the reduction of the biodegradable fraction of organic matter present in the filtered water. Due to the fact that they can also develop in water supply networks, it is very important to effectively disinfect water before it is delivered to consumers.

During the study period, no Enterobacteriaceae bacteria were identified in the water treated in the WTP or in the filter bed samples. This is important because Enterobacteriaceae bacteria may be hazardous to the health of consumers, especially those with impaired immunity.

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Authors' contributions

A. Pruss: the concept of the research, collecting results and their interpretation, the concept of the publication, writing publication and editing publication; A. Wysocka: collecting samples of water and active carbon, making physicochemical and microbiological analyses, interpretation of the results, writing and editing publication; P. Kołaski: collecting samples of water and active carbon, physicochemical and microbiological analyses; I. Lasocka-Gomuła: collecting samples of water and active carbon; M. Michałkiewicz: the interpretation of microbiological analyses; Z. Cybulski: the identification of the bacterial species.

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