Archives of Environmental Protection
Vol. 47 no. 3 pp. 37–49

PL ISSN 2083-4772
DOI 10.24425/aep.2021.138462

The application of different methods for indirect microbial development assessment in pilot scale drinking water biofilters

Dorota Holc[1], Beata Mądrecka-Witkowska[1], Małgorzata Komorowska-Kaufman[1]*, Elżbieta Szeląg-Wasielewska[2], Alina Pruss[1], Zefiryn Cybulski[3]

[1]Poznan University of Technology, Institute of Environmental Engineering and Building Installations, Poland
[2]Adam Mickiewicz University in Poznań, Faculty of Biology, Department of Water Protection, Poland
[3]Greater Poland Cancer Center, Microbiology Laboratory, Poland

*Corresponding author's e-mail: malgorzata.komorowska-kaufman@put.poznan.pl

Keywords: biofilm, drinking water treatment, pilot scale, microbiological activity, biological activated carbon filters (BAC), identification of microorganisms.

Abstract: The biofiltration process in the biologically activated carbon filters (BAC) is one of advanced methods of water treatment. It enables efficient elimination of dissolved organic matter and some inorganic pollutants. The production of high-quality drinking water requires an appropriate method of filter work control based on biofilm growth assessment. The first aim of the study was to assess the microbial development in beds of two BAC filters with the use of various methods. The second aim was to compare the obtained results and indicate the method which could support filter operators during routine control of biofiltration process. The study was carried out in a pilot scale on models of BAC filters during two filter runs. The analysis of microorganisms was performed in water samples collected from different depths of the filter beds with the use of culture method (HPC), metabolic activity assay (with the FDA), epifluorescence microscopy – total cell count method (TCC) and biochemical method (system Vitek 2 Compact). No statistical correlation between HPC and metabolic activity assay was noted. Total bacteria number determined with the use of TCC was approx. 100–900 times higher than in the HPC method. The biochemical tests revealed the presence of several Gram-negative species. The comparison of the applied methods shows that microbial activity assay is the most useful, fast and low-cost method which may be applied additionally to the HPC method at standard water treatment plant laboratory.

Introduction

The production and supplying safety water by water treatment plant is based on effective processes of the removal of any microorganisms, parasites or substances in number or concentration harmful for human health. The requirements for drinking water constantly increase. The list of pollutants that should be reduced during water treatment grows as scientists discover their detrimental effect on human and the natural environment as well as develop the methods for their determination and elimination. In the result of the revised Drinking Water Directive formally adopted by the European Parliament at the end of 2020, the new parameters of water quality will have to be tested and lowered to reach the parametric values recommended in "the Directive" by the water supply entities in the European Union in the near future. These parameters include, e.g., microplastic, per- and polyfluoroalkyl substances (PFASs), pharmaceuticals and endocrine-disrupting compounds (Directive (EU) 2020/2184).

One of water treatment technologies which can be involved in the elimination of a great number of mentioned pollutants from drinking water is a biofiltration. It is a process that has been used at modern water treatment plants for decades. It enables the efficient removal of many organic chemical substances known as dissolved organic matter (DOM) (Pruss et al. 2009, Kaarela et al. 2015, Papciak et al. 2016, Kaleta et al. 2017, Fu et al. 2017) as well as some inorganic chemical compounds, e.g., ammonia (Hasan et al. 2020). This process also helps to ensure the biological stability of water supplied to consumers by the reduction of biogenic substances required to the growth of microorganisms (Szuster-Janiaczyk 2016, Chaukura et al. 2020).

Commonly used filtration medium in the biologically activated filters (BAC) process is a granular activated carbon (GAC), whose properties such as adsorption capacity and large specific surface area (usually 600–1000 m^2) provide excellent conditions for the effective adsorption of chemical compounds as well as for the growth of microorganisms. After the start-up of filter filled with raw GAC only the adsorption

process occurs. It leads to gradual filling the pores of carbon grains with DOM, what induces the microbial colonization. The phase of full acclimation of bacteria lasts for 2–3 months. The biofilm is formed by the bacteria attached to the carbon grains surface as a results of the cell divisions. Bacteria living in biofilm secrete extracellular polymeric substances (EPSs) which act as a matrix stabilizing cells and protecting them against external factors. Biodegradation of DOC occurs as degradation of substances present in the treated water as well as the result of desorption of partially degraded compounds from the GAC pores. The microbial activity in the BAC extends the filtration time and reduces the necessity of activated carbon bed regeneration (Seredyńska-Sobecka et al. 2006, Simpson 2008, Kołwzan 2011).

The filtration trough the BAC filter beds enables great removal efficiency of contaminants of emerging concern, e.g., pharmaceuticals, pesticides (Zang et al. 2017), endocrine disrupting chemicals, personal care products (Snyder et al. 2007), disinfection by-product precursors (Chaukura 2020), cyanotoxins, geosmin and 2-methylisoborneol, amines, aliphatic aldehydes, phenols/chlorinated phenols (Simpson 2008). It can be useful in per- and polyfluoroalkyl ether acids elimination (Hopkins et al. 2018).

The achievement of the benefits of BAC application in water treatment technology depends on the appropriate methods of filter work's control. The effects of biofiltration process are affected by many factors such as the quality of the treated water, water temperature, DO and pH level, flow velocity, the stage of biofilm growth, backwashing frequency (Korotta-Gamage and Sathasivan 2017). The number, activity or biodiversity of microorganisms living in the bed can be studied by many methods, e.g., culture method – heterotrophic plate count (HPC), Eberhardt, Madsen and Sontheimer (EMS) test, the determination of microbial activity with the use of specific chemical compounds, e.g., fluorescein diacetate (FDA) or 2,3,5-triphenyltetrazolium chloride (TTC), the quantification of adenosine triphosphate (ATP), the use of glucose-labeled ^{14}C, the observation with the use of microscopic techniques, the determination of chemical compounds of the biofilm (phospholipids, polysaccharides and proteins) and biochemical tests (Mądrecka et al. 2018). Nowadays, also the techniques of molecular biology are more frequently used to discover and describe the taxonomical diversity and functioning of microorganisms living in a biofilm of BAC, e.g., polymerase chain reaction (PCR) based methods, 16S rRNA analysis, denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) and next generation sequencing (NGS) techniques used in metagenomics (Douterelo et al. 2014). Most of these methods are still too expensive, demand special equipment or qualified laboratory personnel to use them in the routine analyses at water treatment plants. Studies on biofilm formation and functioning with the use of commonly known and old methods as well as comparing them with modern ones are still very useful.

The first aim of the study was to determine and compare the amount, activity and diversity of bacteria growing in beds of two BAC filter columns which differ with the method of bacterial inoculation. Research was conducted in a pilot scale and included two filter runs during the full microbiological activity of BAC. The evaluation of the changes of microbiological activity in the filters was made in water samples taken from different depths of filters bed with the use of traditional culture method (HPC), the metabolic activity assay (with the FDA) and the epifluorescence microscopy techniques. Additionally, taxonomical identification based on biochemical method (system Vitek 2 Compact) was used. The microbiological studies were carried out in water samples collected in the filter bed cross-section and were accompanied by chemical analysis of inflowing and outflowing water.

The second aim of the research was to compare the results obtained from chosen methods and indicate the most useful method, which could support filter operators during routine control of the biofiltration process.

Methods

Research installation

The research was conducted on a pilot scale with the use of physical models of rapid filters (Fig. 1). The research installation consisted of two filter columns – Filter 1 (F1) and Filter 2 (F2). Each filter column's height was 300 cm, inner diameter was 10 cm. and the total filter bed height was 210 cm. The filter medium was activated carbon (WG-12; Gryfskand Ltd. Poland) made of special, low-ash coal, connected by a binder and activated by water vapor characterized by the following parameters: iodine quantity 1,100 mg/g, methylene blue adsorption 30g/100g, specific surface area (BET) 1,100 m^2/g, particle size 1.5–0.75 mm. In the vertical cross-section of each filter column five connectors for collecting bed samples (Z1–Z5) and five connectors for collecting water samples (W1–W5) were installed. The connectors for water samples were placed at the following depths of the beds: 45 cm, 85 cm, 125 cm, 165 cm, and 205 cm. The connectors for bed samples were located at beds' depth: 45 cm, 75 cm, 115 cm, 155 cm and 195 cm. The filter columns were surrounded by a water jacket. The continuous flow of water from the bottom to the top of the water jacket ensured an even temperature throughout the bed's depth. The filter beds were protected against the light and algae growth with a black geotextile cover placed on the filter columns. Both filtration columns were supplied with tap water from the municipal water supply network fed from the oldest WTP, which is currently before modernization due to the high concentration of organic matter (COD=3.37±0,24 mg O_2/dm^3; TOC 4.63±0.26 mg C/dm^3; UV_{254}=0.846±0.58 m^{-1}). At present, water is abstracted from the Warta river, and after a process of artificial infiltration in infiltration ponds it acquires characteristics of the underground water and is therefore aerated and filtered by anthracite-quartz beds of rapid filters and then, after disinfection with UV rays and sodium hypochlorite, directed to the water supply network. Due to the content of organic matter and increasing water stability in the water supply network, the planned modernization of the station will result in the appearance of another barrier, i.e., biologically active carbon filters.

The concentration of residual chlorine was checked several times in the water supplied to the filter model during the study and no residual chlorine was detected.

Filters operation

The main difference between both columns was method of inoculation with bacteria. Filtration in both filters started at the same time. However, F1 from the start was supplied with tap

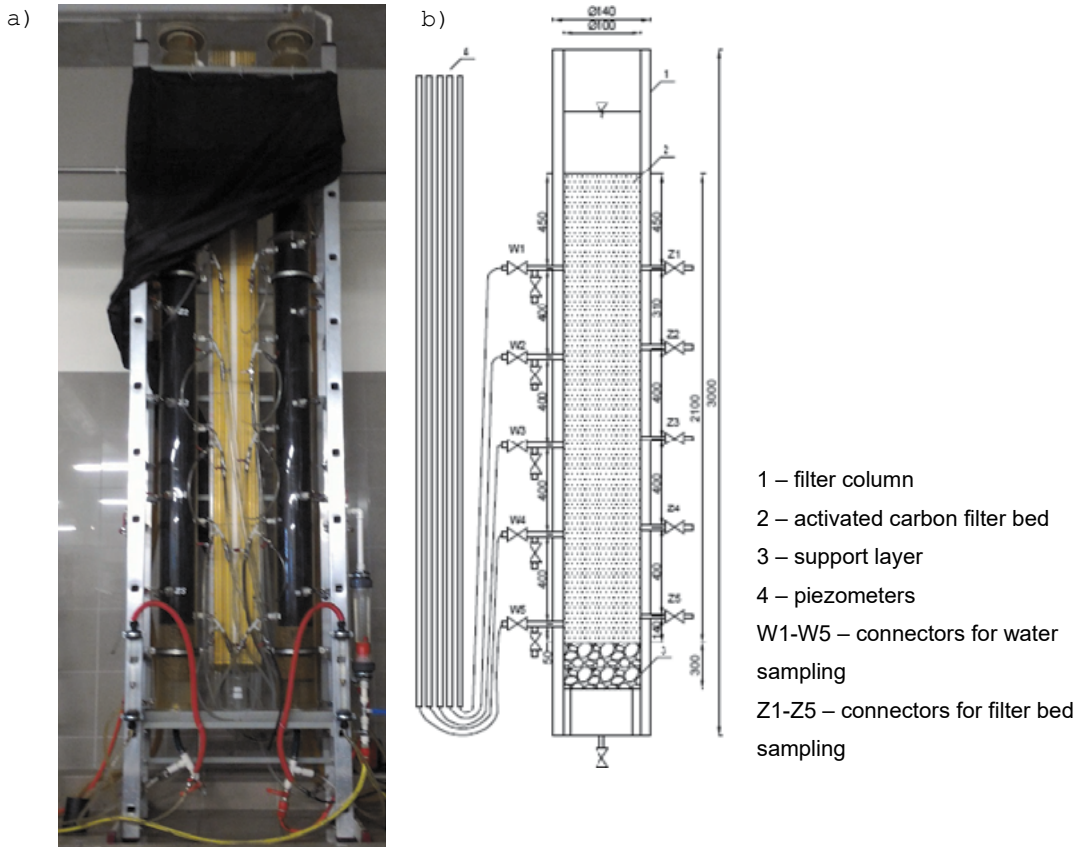

Fig. 1. Research installation a) photography of two filter columns b) construction and dimensions of a single filter column

water, while F2 for first two weeks worked in closed circuit treating backwashings taken from full scale water treatment plant BAC filters diluted with tap water. The backwashings were additionally supplied with nutrients such as ammonium chloride, potassium phosphate and natural organic compounds (onion extract) in order to accelerate the working of the bed (Holc et al. 2016a, Holc et al. 2016b). After the inoculation period F2 was supplied with tap water in the same way as F1. The filters were then used in scientific research and after several weeks an interval in the research started. During this interval, the filters were working with a water flow rate of 20 dm^3/h and were not backwashed for approximately 275 days (run 0), because there was no significant increase in hydraulic losses. After that time, the research described in this article began. It included two runs – both lasting 55 days for each filter. Filters worked in parallel with the weekly delay in backwashing. During two runs both filtration columns were supplied with municipal tap water and operated under the same hydraulic conditions: water flow rate was 40 dm^3/h, the filtration velocity was 5.1 m/h and the bed contact time was 27.2 minutes.

Each filter was backwashed for 17 minutes with tap water at an intensity of 300 L/h, ensuring an expansion of approx. 30% (Komorowska-Kaufman et al. 2018).

Water sampling, laboratory analyses

Water samples for testing physical, chemical and microbiological parameters were taken once a week. The samples for physical and chemical parameters were taken from the filters' inflow and outflow, while the samples for microbiological analyses were collected form five filters' depths.

Physical and chemical parameters included: temperature, pH, dissolved oxygen (DO), alkalinity, UV_{254} absorbance and total organic carbon (TOC). These parameters were analyzed in accordance to the Standard Methods Guidelines (2017). For TOC measurements, a TOC/TN multi NC 3100 (Analityk Jena, Switzerland) was used. The determination of the carbon content was made by thermocatalytic decomposition of sample in the presence of an N/C catalyst at 800°C with synthetic air as the carrier gas. Chemical oxygen demand (COD_{Mn}) was determined by acidic permanganate method according to Polish Standard PN-C-04578-02:1985.

The microbiological analyses involved: heterotrophic place count (HPC), microbial activity (FDA method), total cell count (TCC), and taxonomical identification based on biochemical methods. The heterotrophic plate count (HPC) allowed to determine the number of culturable bacteria. It was performed in accordance to the Polish standard PN-EN ISO 6222:2004. Pour plate technique on enriched agar and incubation at 22°C for 72 hours was applied.

Microbial activity was measured by the determination of esterase activity with the use of FDA. Measurement of FDA hydrolysis rate (fluorescein luminescence intensity) was made on the luminescence spectrometer (PerkinElmer Instruments LS55) and dedicated software (FLWinLab) at the excitation wavelength of 433 nm and the emission wavelength of 525 nm according to the methodology described by Mądrecka et al. (2018).

The representative water samples of the first filter run were studied with the use of TCC method. The selected samples were taken from the depth in which comparable number of bacteria determined by HPC method were found in both filters. The water samples for microscopic analyses were preserved with buffered glutaraldehyde to a final concentration of 10% and stored at 4ºC in the dark. Subsamples of 1 cm^3 were concentrated on black Nuclepore polycarbonate filters with the pore size of 0.2 µm (Whatman) and stained with fluorochrome DAPI (4',6-diamidino-2-phenylindole). Next, the filters were examined with cell secondary fluorescence (Porter and Feig 1980) under UV light excitation and a magnification of 1500× using the Olympus BX-60 epifluorescence microscope equipped with a Jenoptik Gryphax camera and a mercury light source (HBO 100W). In order to determine the number of bacterial cells in each sample, a set of photographs was taken at transects along the diameter of filter. Twenty to twenty five photographs were examined or at least 300 cells were counted for each sample with the use of Olympus Image Analysis System. Measurements of cell dimensions of every counted bacterial cell were also made. Bacteria were classified into recognized morphological forms (cocci, diplococci, coccobacilli, rods, diplobacilli, vibrios, spirilla, spirochaetes, filamentous and trichomes). The cell number was expressed per 1 cm^3. Then the total cell biovolume was estimated on the basis of measurements of the cell size and cell number. The biomass was expressed as wet weight in mg per 1 dm^3, assuming that the biovolume of 10^9 μm^3 is equivalent to 1 mg (Szeląg-Wasielewska et al. 2009).

The taxonomical identification was performed with the automatic Vitek 2 Compact (bioMérieux, Marcy l'Etoile, France) according to the producer's protocol (Pincus 2013). The analysis concerned microbial cultures received by HPC method during two filter runs at each depth of two filters on all sampling dates. Firstly, representative number of colonies from each Petri plate was tested by Gram staining (Smith and Hussey 2016) to know the cell shape and recognise Gram-positive and Gram-negative bacteria. As staining revealed that identified colonies belonged to Gram-negative bacteria, GN Colorimetric Identification Cards (bioMérieux, Marcy l'Etoile, France) were used to perform taxonomical identification. These cards enable to identify Gram-negative fermenting and non-fermenting bacilli, so only this group of bacteria was analysed with biochemical method. The accepted identification confidence (probability) used in this study was ≥93% (very good and excellent confidence levels) (Pincus 2013).

Results

Physical and chemical parameters of water

Filter 1 (F1) and Filter 2 (F2) inflow and outflow water quality during the tests is shown in Table 1 and Table 2, respectively. Generally water inflow to both filtration columns differed slightly due to the weekly delay of the start time of the filtration cycle in F2. Larger differences in water quality were found between the two runs. In the run 2, all analyzed inflow water parameters were slightly lower than in run 1, except for COD_{Mn} and TOC, which were slightly higher, and temperature that was approx. 2.5°C higher than in run 1. During both runs the concentration of ammonia, nitrites and nitrates and orthophosphates in the inflow to both filters was measured randomly (5 times) and ranged from 0.01–0.17 (av.0.07) mg $N\text{-}NH_4/L$, 0.00–0.05 (av.0.02) mg $N\text{-}NO_2/L$, 1.61–2.35 (av.1.88) mg $N\text{-}NO_3/L$ and 0.08–0.44 (av.0.18) mg $P\text{-}PO_4/L$.

As a result of filtration through a biologically active bed, the content of organic compounds in water expressed by COD_{Mn}, TOC concentration and UV_{254} absorbance decreased in both filters. The dissolved oxygen concentration also decreased after flowing through both filters.

The amount of organic matter removed by filtration (ΔCOD_{Mn}) of water through F1 at the end of the run preceding the test (run 0) was equal 0.49 mg O_2/dm^3 and dissolved oxygen consumption (ΔDO) was 1.59 mg O_2/dm^3. In run 1, the filter efficiency decreased and only 0.26±0.18 mg O_2/dm^3 of COD and 1.24±0.59 mg O_2/dm^3 of DO were removed, while in run 2 it increased again to ΔCOD_{Mn} equal 0.54±0.38 mg O_2/dm^3 and ΔDO equal 1.05±0.27 mg O_2/dm^3.

In F2, at the end of run 0 the leaching of the biofilm from the bed had already occurred. The increase in the concentration of COD_{Mn} in the outlet from filter was found, while oxygen was still used for the processes taking place in the bed. ΔDO was similar to that during the entire study and amounted to 1.33 mg O_2/dm^3. In both research runs, the efficiency of F2 was similar. It removed in run 1 and run 2, respectively, 0.49±0.21 mg O_2/dm^3 and 0.50±0.14 mg O_2/dm^3 of COD_{Mn}, and 1.54±0.36 mg O_2/dm^3 and 1.31±0.23 mg O_2/dm^3 of DO. During the run 2 the obtained filtration effects were more stable than in run 1.

The average efficiency of organic compounds removal during the whole research period (run 1 and 2) was as follows: COD_{Mn} 10.25±5.71%, TOC 7.17±10.59% (max 16.00%) and UV_{254} absorbance 31.27±18.82% for F1 and COD_{Mn} 14.45±7.23%, TOC 5.57±10.44% (max 20.18%) and UV_{254} absorbance 29.71±10.77% for F2. The removal efficiency of organic pollutants in the filters was very variable during the cycle. The obtained effects indicate that despite the same hydraulic conditions and quality of inflow water, the F2 worked better, which should be justified by the presence of microorganisms from the inoculated backwashings.

Results of HPC method and microbial activity assay Development of microorganisms in the filter during filtration run

Changes in the number of bacteria and microbiological activity in the section of the bed depending on the duration of the filtration run are shown in Fig. 2. Average values obtained from the analysis of water samples taken on a given day in the whole bed profile (connectors 1–5) are presented. It is clearly visible that the number of HPC bacteria in water samples taken at the end of run 0 from both filters and in the whole run 1 of F2 was varied at different depths, which is indicated by a significant standard deviation. The differences in microbial activity measured at different depths were much smaller. Run 1 in both filters showed a higher number of bacteria in the water samples taken from the bed than in run 2, which was a consequence of a very intensive development of biofilm in the previous run, which was carried out for a long time with half of the filtration velocity. The filtration

velocity affects the structure of the biofilm, which becomes more compact at higher velocities (Pruss 2007). With time, all the scattered fragments break away from it and the amount of slowly flowing bacteria decreases (Fig. 2b). Also in F1, during run 1, the number of slow-flowing bacteria in water samples has decreased. Probably in F1 there was lower biofilm volume (biomass concentration removed during rinsing was lower – results not included) than in F2 and the decreasing effect which we recorded in F2 from the 27th day of the cycle started earlier (Fig. 2a). In run 2, in both filters, the number of HPC bacteria in water samples increased with time as the biofilm was developing. It was noticed that the number of bacteria in F2 was always higher than in F1.

The difference in microbial activity in both filter runs in both filters was much greater than the difference in HPC bacteria (Fig. 2c–d). The highest activity was found in run 0 samples. During run 1 in F2 the microbial activity decreased slightly, while in F1 it definitely decreased during the first 40 days and later it almost doubled. During run 2 an increase in the measured microbial activity was recorded in both filters and it was definitely higher for F2.

In order to determine the capacity of bacteria living in the filter to decompose organic compounds, the microbial activity/HPC bacteria indicator was used. It informs about decomposition yield of FDA by one bacteria in water samples from the filter and is called unit activity ((A/min)/CFU). The change of this indicator during the filtration run was similar to the activity (Fig. 2e–f). However, a high variability of this parameter was observed at different depths of the bed.

Development of microorganisms in the filter bed profile

Figure 3 shows changes in the number of HPC bacteria and microbial activity in the profile of the studied filter beds. The

Table 1. The Filter 1 (F1) inflow and outflow water quality during experimental period (δ – standard deviation)

Parameter	Unit	Run 1				Run 2			
		Inflow		Outflow		Inflow		Outflow	
		min – max	average±δ	min – max	average±δ	min – max	average±δ	min – max	average±δ
Temperature	°C	13.4–15.3	14.46± 0.67	14.6–19.0	17.14± 1.53	15.8–18.0	16.80± 0.87	16.7–20.7	18.80± 1.38
pH	–	7.45–8.01	7.82± 0.18	7.40–7.87	7.64± 0.15	7.47–7.88	7.71± 0.15	7.06–7.73	7.49± 0.22
Total alkalinity	mg $CaCO_3/dm^3$	3.72–4.03	3.86± 0.12	3.56–4.01	3.74± 0.14	3.65–3.89	3.78± 0.07	3.51–3.67	3.62± 0.05
Dissolved oxygen	mg O_2/dm^3	4.35–5.56	4.83± 0.43	2.98–4.07	3.59± 0.39	3.96–4.98	4.30± 0.32	2.54–3.91	3.25± 0.40
COD_{Mn}	mg O_2/dm^3	3.06–3.77	3.37± 0.24	2.83–3.36	3.11± 0.18	3.20–4.03	3.68± 0.29	2.99–3.40	3.19± 0.13
TOC	mg C/dm^3	4.40–5.00	4.63± 0.26	4.2	4.20± 0.02	4.36–4.99	4.64± 0.32	3.95–5.00	4.39± 0.54
UV_{254}	m^{-1}	0.62–2.28	0.846± 0.580	0.40–0.60	0.494± 0.006	0.61–1.22	0.760± 0.195	0.39–0.75	0.514± 0.113

Table 2. The Filter 2 (F2) inflow and outflow water quality during experimental period (δ – standard deviation)

Parameter	Unit	Run 1				Run 2			
		Inflow		Outflow		Inflow		Outflow	
		min – max	average±δ	min – max	average±δ	min – max	average±δ	min – max	average±δ
Temperature	°C	13.4–16.1	14.56± 0.87	16.4–20.2	19.14± 1.21	14.6–19.4	16.99± 1.58	17.9–21.0	19.49± 1.13
pH	–	7.55–8.01	7.85± 0.14	7.44–7.61	7.55± 0.06	7.54–7.84	7.70± 0.12	7.32–7.63	7.48± 0.12
Total alkalinity	mg $CaCO_3/dm^3$	3.72–3.97	3.82± 0.09	3.55–3.99	3.72± 0.15	3.61–3.89	3.77± 0.09	3.52–3.70	3.60± 0.07
Dissolved oxygen	mg O_2/dm^3	4.35–5.56	4.81± 0.48	2.98–3.98	3.27± 0.36	3.96–4.64	4.25± 0.24	2.57–3.61	2.94± 0.33
COD_{Mn}	mg O_2/dm^3	3.06–3.99	3.41± 0.34	2.51–3.17	2.87± 0.24	3.20–4.16	3.73± 0.35	2.70–3.82	3.23± 0.40
TOC	mg C/dm^3	4.40–5.00	4.58± 0.25	4.20–4.90	4.36± 0.30	4.36–5.00	4.74± 0.31	3.48–4.99	4.42± 0.71
UV_{254}	m^{-1}	0.63–0.67	0.645± 0.013	0.38–0.58	0.468± 0.058	0.67–1.22	0.770± 0.200	0.39–0.61	0.507± 0.073

average values obtained from the analysis of water samples taken from the depth of 45, 85, 125, 165 and 205 cm for the entire cycle are presented. The greatest differences in the values of the measured parameters were recorded on the last day of run 0 that preceded the research period. In the tested runs, the average number of bacteria grown from the water samples did not change unambiguously with depth (Fig. 3a–b).

Detailed analysis showed that it is difficult to determine a clear trend because in the following days of the filtration run the number of bacteria in the samples from the bed profile both increased and decreased, or changed irregularly. In run 1, however, it is possible to determine the depth at which the greatest number of bacteria was usually determined, i.e., 165 cm in F1 and 125 cm in F2. In run 2 in the F1 filter bed, the number of bacteria in water samples decreased slightly with depth. In the F2 filter bed, it decreased at the depth of 85 cm and increased slightly later.

In F1, the highest microbiological activity in the samples of water collected in the bed profile was recorded at the end of run 0 (Fig. 3c–d). In subsequent runs, it was approximately constant throughout the depth of the bed, although there were significant fluctuations in activity in the samples from different bed depths in the consecutive days of the run, similar to the amount of HPC bacteria. In run 1, it was slightly higher than in run 2. In the F2 bed, in run 1, the microbiological activity was comparable to that in F1 bed, while in run 2, it was much higher and slightly decreased with depth.

The microbial activity/HPC bacteria indicator reached its highest value in F1 in run 0 at the depth of 85 cm. In the remaining runs, up to the depth of 125 cm, an increase in the unit

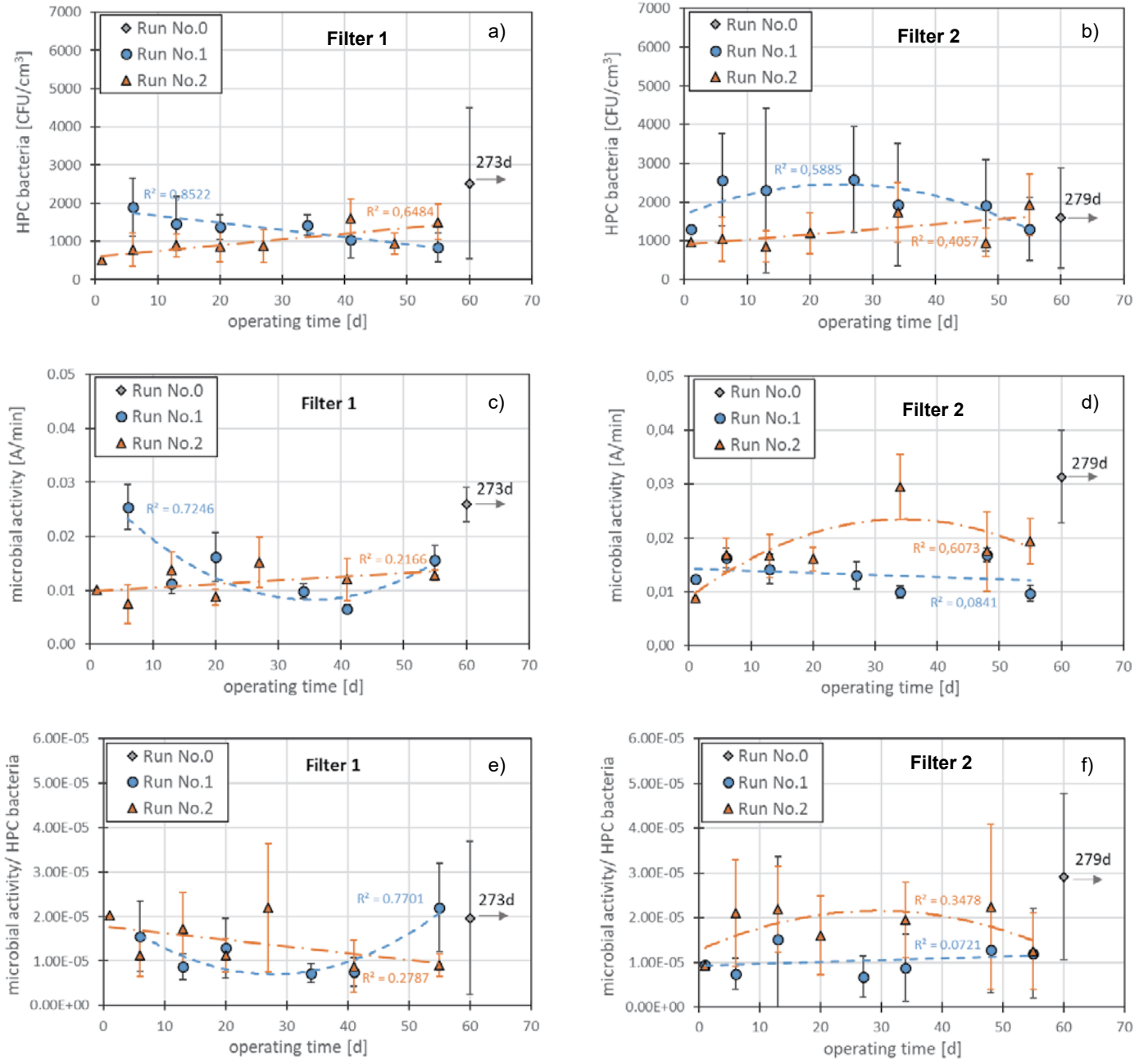

Fig. 2. Change of average values of HPC bacteria (a,b), total microbial activity (c,d) and unit water activity (e,f) in water samples taken from the filter F1 (a,c,e) and filter F2 (b,d,f) bed during filter run

activity of bacteria was found. While below this depth in run 1, a decrease in unit activity was observed, and in run 2, its further increase was noted. The F2 also showed the highest unit activity in run 0 at the depths of 85 and 125 cm. Also in the remaining two runs, up to the depth of 85 cm, the unit activity increased, later it started to decrease and stabilized at the depth below 165 cm. In the entire profile of the F2 bed, higher unit activity was found in run 2 than in run 1, which confirms that proper backwashing of excess biofilm allows for the development of appropriate bacteria and better diffusion of nutrients.

Relationship between HPC and microbial activity in BAC

No correlation was found between the number of cultured bacteria and microbial activity determined by the FDA method in all analyzed water samples taken from different heights of the deposit on different days of the cycle (Pearson correlation coefficient $R < 0.3$) (Fig. 4). The spread of both HPC bacteria and microbial activity was greater in F2. Much more bacteria that did not show proportionally high activity were found in filter F2 in run 1. This may confirm the assumption that these bacteria come from fragments of "old biofilm" left over from backwashing.

Total cell count method

The total bacteria number and biomass determined with the use of an epifluorescence microscope in the water samples collected from filter bed were changing during the filters' run (Table 3, Fig. 5). Before the first filter backwashing, at the end of run 0, after about 275 days of continuous filtration,

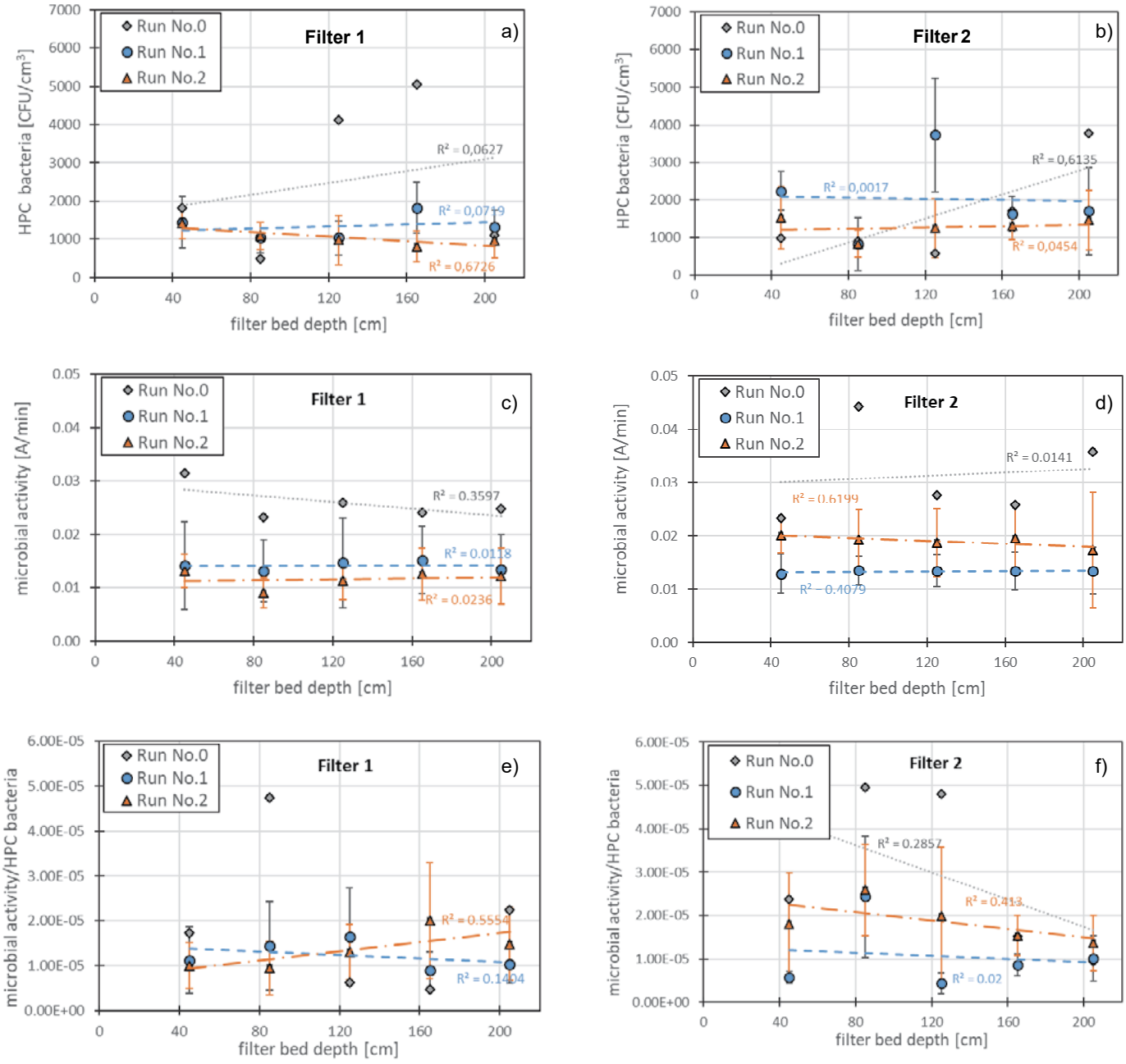

Fig. 3. Change of average values of HPC bacteria (a, b), total microbial activity (c, d) and unit water activity (e, f) in water samples taken from different filter bed depths of filter F1 (a, c, e) and filter F2 (b, d, f) bed during filter run

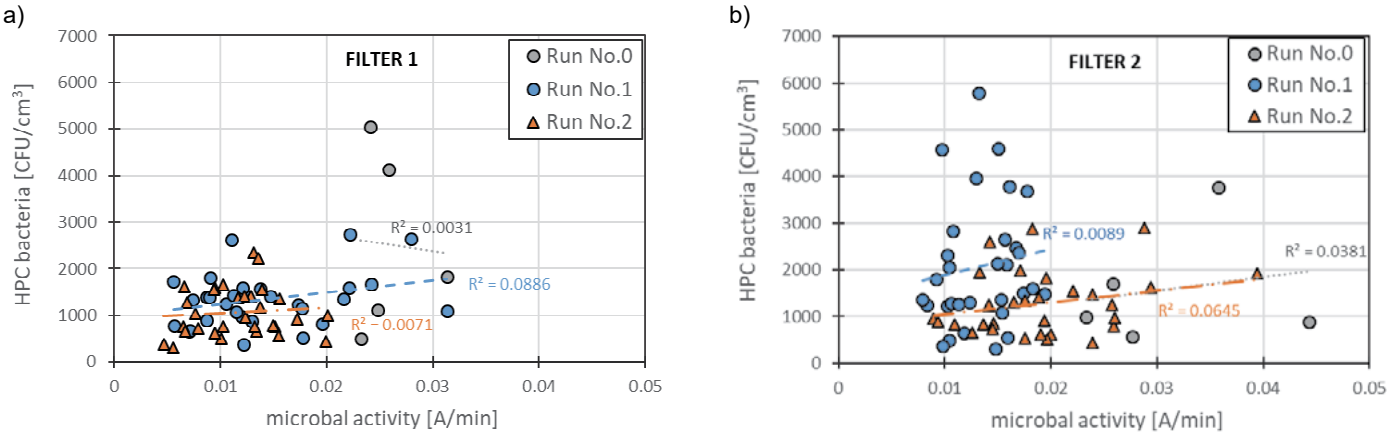

Fig. 4. The relationship between microbial activity and the number of HPC bacteria in water samples from the entire filter (all layers) a) F1 b) F2

Table 3. Cell number and biomass (expressed as wet weight) of bacteria and their morphological forms in selected water samples of F1 and F2.

Parameter	Filter no and filter depth	Run / operating time (day)					
		Run 0		Run 1			Run 2
		273	278	13	34	55	6
Cocci (10^3 cells/cm^3)	F1 – 125 cm	1475.7	NA	79.9	93.8	109.4	124.8
	F2 – 165 cm	NA	247.2	68.2	110.8	NA	159.8
Diplococci (10^3 cells/cm^3)	F1 – 125 cm	10.6	NA	2.1	0.0	2.8	0.0
	F2 – 165 cm	NA	4.3	0.0	4.3	NA	10.7
Coccobacilli (10^3 cells/cm^3)	F1 – 125 cm	314.3	NA	6.4	33.0	28.4	30.4
	F2 – 165 cm	NA	75.6	20.2	22.4	NA	25.6
Rods (10^3 cells/cm^3)	F1 – 125 cm	873.7	NA	66.1	65.0	32.7	91.3
	F2 – 165 cm	NA	184.3	38.3	27.7	NA	101.4
Diplobacilli (10^3 cells/cm^3)	F1 – 125 cm	21.3	NA	0.0	0.0	0.0	0.0
	F2 – 165 cm	NA	2.1	2.1	2.1	NA	8.5
Vibrios (10^3 cells/cm^3)	F1 – 125 cm	623.3	NA	27.7	34.1	35.5	39.6
	F2 – 165 cm	NA	109.7	27.7	32.2	NA	20.5
Spirilla (10^3 cells/cm^3)	F1 – 125 cm	42.6	NA	0.0	0.0	1.4	0.0
	F2 – 165 cm	NA	12.8	1.1	9.6	NA	5.3
Spirochaetes (10^3 cells/cm^3)	F1 – 125 cm	218.4	NA	0.0	0.0	2.8	57.8
	F2 – 165 cm	NA	0.0	0.0	1.1	NA	12.8
Filamentous (10^3 cells/cm^3)	F1 – 125 cm	63.9	NA	0.0	0.0	5.7	3.0
	F2 – 165 cm	NA	5.3	0.0	0.0	NA	1.1
Trichomes (10^3 cells/cm^3)	F1 – 125 cm	0.0	NA	0.0	0.0	0.0	0.0
	F2 – 165 cm	NA	1.1	0.0	0.0	NA	1.1
Total cell number (10^3 cells/cm^3)	F1 – 125 cm	3644.0	NA	182.2	225.9	218.8	347.0
	F2 – 165 cm	NA	642.5	157.7	209.9	NA	349.7
Total cell biomass (mg/dm^3)	F1 – 125 cm	1.092	NA	0.021	0.026	0.017	0.044
	F2 – 165 cm	NA	0.139	0.031	0.012	NA	0.060

NA – not analyzed

the total number and biomass of bacteria were several times higher in F1 than in F2 (about 6 times in the case of bacteria number and about 8 times in the case of the biomass). The first backwashing led to biofilm separation and washing it out of the beds. In the result of backwashing, on the 13th day of filters run 1, the bacterial number and biomass decreased. The total cell number was similar in both filters, but the biomass was lower in F1 than in F2. On the 34th day of the run 1, the bacterial number and biomass increased what indicated the biofilm growth. The bacterial number in F1 was only slightly higher than in F2, while the biomass was two times higher than in F2. The higher bacterial biomass was the result of higher

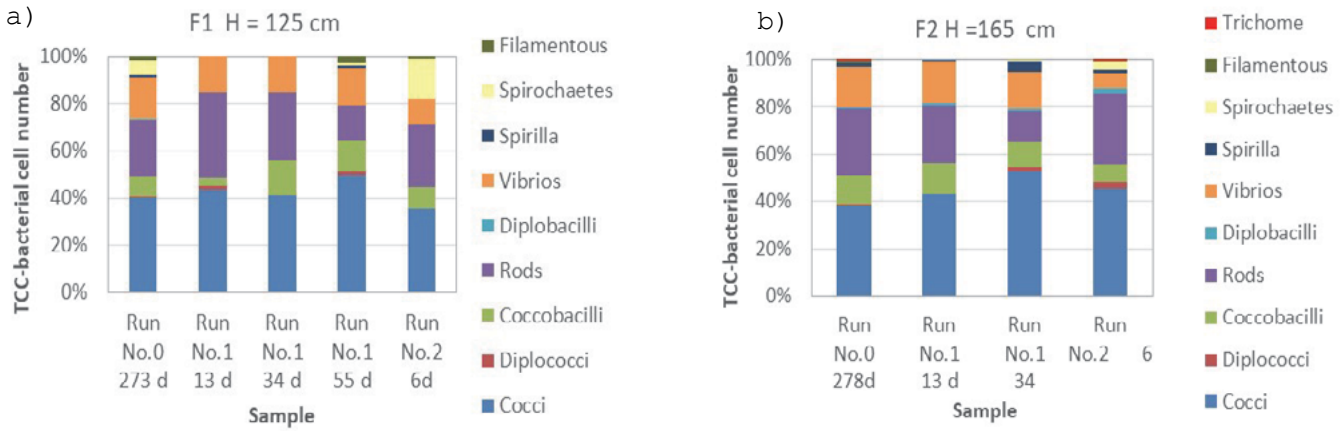

Fig. 5. The percentage share of bacterial shapes and arrangements in the total cell number determined with TCC method in selected water samples

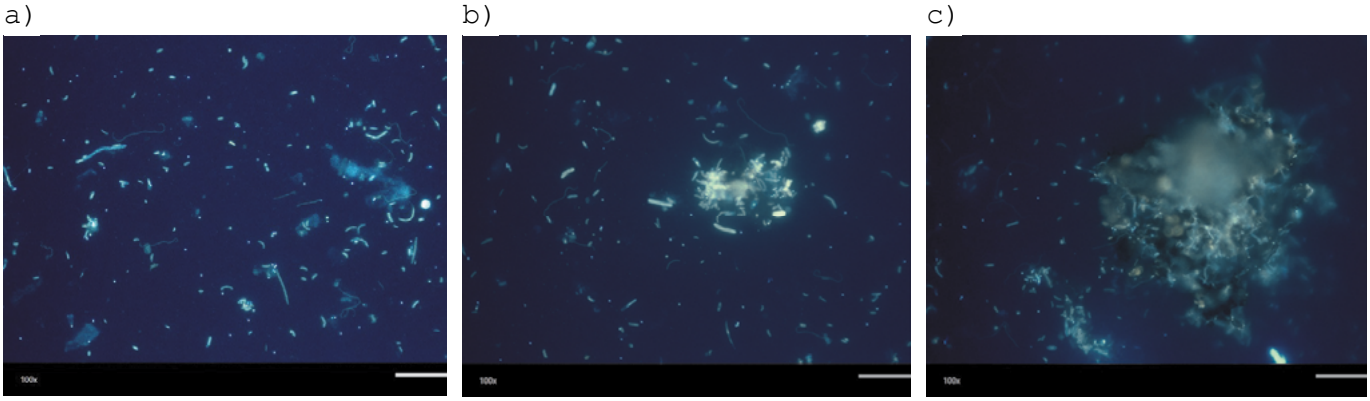

Fig. 6. Bacteria observed under epifluorescence microscope (100×): a) morphological diversity of free-floating bacteria; b) biofilm fragments c) biofilm developed on the carbon grain

number of morphological forms characterized by the large size of cells, e.g., rods. After the second backwashing, on the 6th day of the run 2, the total cell number and biomass were higher than before the second backwashing. On that day the total cell number was similar in both filters but the total biomass in F1 was about 25% smaller than in F2.

The analysis made with the use of TCC method has shown the differences between F1 and F2 in the structure of morphological forms (Table 3, Fig. 5, Fig. 6). The number of spirochaetes, filamentous forms and vibrios were higher in F1 than in F2. The number of cocci, diplococci and rods was changing in both filters, but at the end of the investigated period it was higher in F2. The differences in the morphological structure of bacterial cells indicated that the taxonomical structure of bacterial community developed in both filters was also different. The biomass of free-flowing bacteria derived from the biofilm was changing during filter runs in the range of 0.012–1.092 mg/dm^3. The total bacteria number determined with the use of an epifluorescence microscope was approximately 100–900 times higher than that obtained by HPC method.

Taxonomical identification based on biochemical method – Vitek 2 Compact

The biochemical identification performed with the use of Vitek 2 Compact resulted in the recognition of several bacterial species. The most common species noted in both filter columns during two filter runs was *Stenotrophomonas maltophilia.* Other identified taxa were: *Brevundimonas diminuta/vesicularis*, *Shewanella putrafaciens*, *Sphingomonas paucimobilis* and *Pseudomonas putida*. The last mentioned species was detected only in F2 during the run 2.

Identified species are Gram-negative bacteria preferring mesophilic environment and usually noted as rods. Most of them are aerobic except for *S. putrafaciens*, which is facultative anaerobe. They are mainly noted in natural environment such as water, soil or rhizosphere, but some of them are also isolated from food or are part of animals' microflora. They can be also opportunistic human pathogens, mainly related to the nosocomial infections and observed in immunocompetent patients. They are able to metabolise various inorganic or organic compounds. Strains of the most often noted species *S. maltophilia* are chemoorganoheterotrophic and they use limited range of organic compounds as main carbon and energy source, e.g., glucose, mannose, maltose, cellobiose, acetate, propionate, fumarate, lactate, citrate, l-alanine, d-alanine, l-glutamate, l-histidine, and l-proline. *Brevundimonas diminuta/vesicularis* is chemoorganotrophic and oligotrophic taxon. Its nutritional spectrum is also restricted. It utilizes, e.g. pyruvate and organic acids. *Shewanella putrafaciens* strains can form hydrogen sulfide from various sulfur compounds. It is also known that this bacterium can reduce ferric, manganese and other metals. *Sphingomonas paucimobilis* degrades aromatic hydrocarbons, e.g., it can assimilate betaine, D(+)galactose, l-aspartate, succinate, p-hydroxybenzoate, cis-aconitate, D(+)trehalose,

trans-aconitate, L(-)malate, and l-serine. A distinctive feature of *Pseudomonas putida* is ability to produce siderophores called pyoverdines. It is a fluorescent species, which is noted mainly in soil and water. It can metabolize a widely spectrum of organic compounds as a source of carbon (Garrity 2005a, 2005b). A diverse metabolism is a reason why *P. putida* is used or considered to be useful in bioremediation of various pollutants (Zamule et al. 2021).

Discussion

The presented research concerns the BAC filters, which have been operated for more than 9 months since the start-up and microbial inoculation. The chemical analysis of inflowing and outflowing water revealed average efficiency of organic compounds removal during the two analyzed filter runs – max 16.00% of TOC in the case of F1, and max 20.18% in the case of F2. These data indicate that probably the adsorption capacity of GAC was exhausted and the DOM removal was based mainly by biological degradation (Simpson 2008). However, it is worth noting that TOC removal efficiency of F2 was greater, what can be explained by the colonization with the microorganisms from the backwashings derived from existing water treatment plant. The results of previous studies carried out in a pilot scale have proved that inoculation of the BAC filter with backwashings accelerates the carbon filter activation (Holc et al. 2016a, 2016b). The results of TCC method showed differences in the composition of morphological structure of bacterial population living in two analyzed BAC filters. Biochemical identification carried out with the use of Vitek 2 Compact also indicates some taxonomical differences of microorganism growing in the filters biofilms. These differences may results in more effective working of F2. The removal of organic matter from water is a result of oxidation in the respiratory processes of microorganisms and the increase of their biomass. The decrease in oxygen concentration and the following increase in carbon dioxide concentration in the treated water indicate the development of microorganisms in filter bed (Pruss et al. 2009, Liao et al. 2012, Elhadidy et al. 2017, Kołaski et al. 2019). These findings have been confirmed in the presented studies. The removal of oxygen in F1 (0.49±0.21 mg O_2/dm^3 in run 1 and 0.50±0.14 mg O_2/dm in run 2) was evidently smaller than in F2 (1.54±0.36 mg O_2/dm^3 in run 1 and 1.31±0.23 mg O_2/dm^3 in run 2).

The comparison of HPC method and microbial activity assay revealed no correlation between the number of cultivated bacteria and microbial activity in both BAC filters. Moreover, the results of third analytical method – TCC showed that the total bacteria number determined with the use of an epifluorescence microscope was approximately 100–900 times higher than the number of colony forming units obtained from the HPC method. It means that only about 0.001–0.01% of bacterial cells counted with the use of microscopic techniques was able to grow on culture medium. These values might be overestimated because one colony is the result of binary division of one cell or cell arrangement. However, the results confirm poor detectability of microorganisms with the use of culture methods.

All methods used in the research have advantages and disadvantages. The HPC is an old and easy method of determining a number of culturable heterotrophic microorganisms that can be carried out in a standard microbiological laboratory. This method enables the calculation of the number of various taxonomical or ecological groups of microorganisms. Its main disadvantages can be quite long time of bacterial incubation in some cases or difficulties in taxonomical identification of microorganisms. Its main disadvantage is that only a small percentage of bacteria living in various environments are able to grow on culture media in the laboratory conditions – only 0.01% of waterborne bacteria (WHO 2003), up to 15% of bacteria of activated sludge (Wagner et al. 1993), and 0.05–8.3% of bacteria occurring in drinking water (Burtscher et al. 2009) The methods of microbial cultivation have to deal with the impossibility of recreating a wide range of ecological factors affecting microorganisms in their natural environment. Many bacteria are also able to reduce their metabolism and enter the "viable but nonculturable state" (VBNC), e.g., as a result of environmental stress during samples collection. Bacteria in VBNC state are still alive but not detectable with the use of culture methods (Oliver 2010). Despite these deficiencies, the HPC method is still recommended by law regulation related to the drinking water quality in the European Union (Drinking Water Directive), United States, Canada or Australia (Van Nevel et al. 2017).

The second analyzed method – the measurement of microbial activity using the FDA is based on the determination of esterase activity (Olszewska and Łaniewska-Trokenheim 2013, Lis et al. 2016). Esterase is an enzyme which converts fluorescein diacetate to fluorescein – a green fluorescent chemical compound. Only live cells are capable of showing fluorescence. The advantages of this method are simplicity, low cost and shorter time of performance than HPC method. This method is commonly used to measure the activity of bacteria originating from the natural ecosystems such as soil (Adam and Duncan 2001) and freshwater (Battin 1997) or developing in the man-made environment, e.g., activated sludge (Kijowska et al. 2001, Ziglio et al. 2002). However, some studies have shown that the ability to absorb fluorescein diacetate depends on the taxonomical features and stage of cell growth (Chrzanowski et al. 1984).

The next method applied in the research – total cell count method (TCC) is more sophisticated but can be used to accurately determine the number of microorganisms. It involves epifluorescence microscopy techniques and fluorochromes – the fluorescent dyes that stain cell compounds. The method allows to count bacterial cells, describe their morphology, measure the cell size, and in some cases also recognize their physiological state. The main dyes used for bacteria observation are, e.g., acridine orange, 4',6-diamidino-2-phenylindole (DAPI), propidium iodide, ethidium bromide, PO-PRO-3 and SYTOX Green or the a set of SYTO®9 and PI dyes known as LIVE/DEAD® BacLightTM Bacterial Viability Kit which enable to distinguish the live and dead bacterial cells (McFeters et al. 1995, Boulos et al. 1999, Sadowska and Grajek 2009). Although the determination of bacteria cell number by this method may be very accurate, it is susceptible to errors if bacteria cells are formed in clusters or are attached to microscopic fragments of carbon grains (Van der Kooij et al. 2014). It is also more time-consuming and difficult in performance than the two above mentioned methods.

Biochemical tests are usually based on the determination of immunological or biochemical properties of microorganisms which allow the taxonomical identification, e.g., analytical profile index (API) tests or system Vitek 2 Compact (bioMérieux). The use of biochemical tests is an expensive method, it requires special laboratory equipment and commercial test, but is easy to perform. However, it should be noted that the number of taxa that can be identified by this method is limited. Such tests are mainly designed to be used in, e.g., medical analysis to detect pathogenic and opportunistic microorganisms. Less common and non-human taxa may remain unidentified or misidentified (Pincus 2013).

The results of the presented study and above mentioned benefits as well as deficiencies of biofilm growth assessment indicate that despite the HPC method is still required to perform by law regulation in many countries worldwide, the application of additional method of biofilm growth control gives valuable information. One of these methods is the measuring of microbial activity. It is easy to carry out in the standard laboratory, no time-consuming and low-cost method.

Conclusions

Microbiological analysis of water samples using HCP method, epifluorescence microscope and the microbiological activity test (FDA) showed microbiological activity of both carbon filters. The results of the HPC and FDA test in water samples taken from the vertical profile of the filtration columns confirm that the filter bed was biologically active over its entire depth. Comparing the results for both columns, higher activity values were observed in the F2 column, which indicates more developed biofilm, possibly as a result of earlier inoculation of the bed. The activity of the filtration column was very unevenly distributed in the bed depth. The analysis of the results showed that the backwashing of the filter beds of both columns caused only a slight decrease in microbial activity in the filters, which does not adversely affect the entire cycle. Backwashing, on the other hand, plays an important role in controlling the amount of free-flowing bacteria and the thickness of the biofilm, which has an impact on the results of water treatment.

The study did not show any correlation between the number of HPC bacteria and microbiological activity measured by FDA method. Similarly, no correlation was observed between these parameters in samples of deposits of biologically active filters operated on a technical scale (Kołaski et al. 2019).

Studies have shown that the use of an epifluorescence microscope to determine the counts of heterotrophic bacteria allows to determine the actual counts of bacteria, including those incapable of growing on culture media. The total bacteria number determined with the use of an epifluorescence microscope was approximately 100–900 times higher than the one obtained using the HPC method.

The bacteria identified in the water samples belong to Gram-negative strains that use carbon from the decomposition of organic compounds.

Despite its imperfection, the use of HPC method for continuous testing of the microbial community of BAC filter allows to assess the stage of biofilm formation It helps to control biofilm growth and effective removal of contaminants in BAC filters. Although the TCC method may determine the bacterial cell number very precisely, it is time-consuming, it requires special laboratory equipment and trained personnel to perform. The biochemical methods may give the additional information about the taxonomical structure or changes of the microorganisms in the biofilms but they contain a limited list of microorganisms that can be identified by the design tests. The results of research show no possibility of implementation of TCC methods for the BAC operation.

The comparison of various methods shows that microbial activity assay with the use of FDA is the most useful, no time-consuming and low-cost method which can be applied at standard laboratory at water treatment plant. It provides valuable information which can support BAC filters operators and be used as a routine control of the biofiltration process additionally to the HPC method required by the law regulations.

In the next stage of the research, the evaluation of the biofilm development directly on the grains of the filter beds will be presented, using the same methods and additionally with the use of a confocal microscope. It will also be indicated whether there is a relationship between the indirect and direct methods of assessing the biofilm development in the filter.

Acknowledgement

This research was financially supported by the National Science Centre, Poland; project number 2019/03/X/NZ9/01051 "Determination of the possibility of antibiotic-resistant bacteria growth in biologically active carbon filters used in processes of water treatment" and research projects 504101/0713/SBAD/0936 "Removal of organic matter from water in water treatment processes" and 504101/0713/SBAD/0933 "Research on the microbiome of biologically active carbon filters (BAF) used in water treatment processes".

References

Adam, G. & Duncan, H. (2001). Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biology & Biochemistry*, 33, 7–8, pp. 943–951, DOI: 10.1016/S0038-0717(00)00244-3.

Battin, T.J. (1997). Assessment of fluorescein diacetate hydrolysis as a measure of total esterase activity in natural stream sediment biomass. *The Science of the Total Environment*, 198, 1, pp. 51–60, DOI: 10.1016/S0048-9697(97)05441-7.

Boulos, L., Prévost, M., Barbeau, B., Coallier, J. & Desjardins, R. (1999). LIVE/DEAD® *Bac*Light™: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. *Journal of Microbiological Methods*, 37, 1, pp.77–86, DOI: 10.1016/s0167-7012(99)00048-2.

Burtscher, M.M., Zibuschka, F., Mach1, R.L., Lindne, G. & Farnleitner, A.H. (2009). Heterotrophic plate count vs. in situ bacterial 16S rRNA gene amplicon profiles from drinking water reveal completely different communities with distinct spatial and temporal allocations in a distribution net. *Water SA*, 35, 4, pp. 495–504, DOI: 10.4314/wsa.v35i4.76809.

Chaukura, N., Marais, S.S., Moyo, W., Mbali, N., Thakalekoala, L.C., Ingwani, T., Mamba, B.B., Jarvis, P. & Nkambule, T.T.I. (2020). Contemporary issues on the occurrence and removal of disinfection byproducts in drinking water – A review, *Journal of Environmental Chemical Engineering*, 8, 2, 103659, DOI: 10.1016/j.jece.2020.103659.

Chrzanowski, T.H., Crotty, R.D., Hubbard, J.G. & Welch, R.P. (1984). Applicability of the fluorescein diacetate method of detecting active bacteria in freshwater. *Microbial Ecology*, 10, 2, pp.179–185, DOI: 10.1007/BF02011424.

Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption.

Douterelo, I., Boxall, J.B., Deines, P., Sekar, R., Fish, K.E. & Biggs, C.A. (2014). Methodological approaches for studying the microbial ecology of drinking water distribution systems, *Water Research* 65, pp. 134–156, DOI: 0.1016/j.watres.2014.07.008.

Elhadidy, A.M., Van Dyke, M.I., Chen, F., Peldszus, S. & Huck, P.M. (2017). Development and application of an improved protocol to characterize biofilms in biologically active drinking water filters, *Environ. Sci. Water Res. Technol.*, 3, pp. 249–261, DOI: 10.1039/C6EW00279J.

Fu, J., Lee, W.-N., Coleman, C., Nowack, K., Carter, J. & Huang, C.-H. (2017). Removal of disinfection byproduct (DBP) precursors in water by two-stage biofiltration treatment. *Water Research*, 123, pp. 224–235, DOI: 10.1016/j.watres.2017.06.073.

Garrity G.M. (ed.) (2005a) Bergey's Manual of Systematic Bacteriology. Vol. 2 The *Proteobacteria*, part B The *Gammaproteobacteria*, Springer, New York.

Garrity G.M. (ed.) (2005b) Bergey's Manual of Systematic Bacteriology. Vol. 2 The *Proteobacteria*, part C The *Alpha-Beta-*, *Delta-* and *Epsilonproteobacteria*. Springer, New York.

Hasan, H.A., Muhammad, M.H. & Ismail, N.I. (2020), A review of biological drinking water treatment technologies for contaminants removal from polluted water resources, *Journal of Water Process Engineering*, 33, 101035, DOI: 10.1016/j.jwpe.2019.101035.

Holc, D., Pruss, A., Michałkiewicz, M. & Cybulski Z. (2016). Acceleration of carbon filters activation – experiments of pilot scale technological investigations. *Water supply and water quality.* PZITS, Poznań, pp. 683–703. (in Polish)

Holc, D., Pruss, A., Michałkiewicz, M. & Cybulski Z. (2016). Effectiveness of organic compounds removing during water treatment by filtration through a biologically active carbon filter with the identification of microorganisms. *Annual Set The Environment Protection*, 18, 2, pp. 235–246. (in Polish)

Hopkins, Z.R., Sun, M., DeWitt, J.C. & Knappe, D.R.U. (2018). Recently Detected Drinking Water Contaminants: GenX and Other Per-and Polyfluoroalkyl Ether Acids. *Journal-American Water Works Association*, 110, 7, pp. 13–28, DOI: doi.org/10.1002/awwa.1073.

Kaarela, O. E., Harkki, H. A., Palmroth, M. R. T. & Tuhkanen T. A. (2015). Bacterial diversity and active biomass in full-scale granular activated carbon filters operated at low water temperatures, *Environmental Technology*, 36, 5–8, pp. 681–692, DOI: 10.1080/09593330.2014.958542.

Kaleta, J., Kida, M., Koszelnik, P., Papciak, D., Puszkarewicz, A. & Tchórzewska-Cieślak B. (2017). The use of activated carbons for removing organic matter from groundwater, *Archives of Environmental Protection*, 43, 3, pp. 32–41, DOI:10.1515/aep-2017-0031.

Kijowska, E., Leszczyńska, M. & Sozański, M.M. (2001): Metabolic activity test in investigation of biodegradation in biological filters, *Water*, *Science & Technology: Water Supply*, 1, 2, pp. 151–158, DOI: doi.org/10.2166/ws.2001.0032.

Kołaski, P., Wysocka, A., Pruss, A., Lasocka-Gomuła, I., Michałkiewicz, M. & Cybulski Z. (2019). Removal of Organic Matter from Water During Rapid Filtration through a Biologically Active Carbon Filter Beds – a Full Scale Technological Investigation, *Annual Set The Environment Protection*, 21, 2, pp. 1136–1155.

Kołwzan, B. (2011). Analysis of biofilms – their formation and functioning. *Environmental Pollution Control*, 33, 4, pp. 3–14. (in Polish)

Komorowska-Kaufman, M., Ciesielczyk, F., Pruss, A. & Jesionowski T. (2018). Effect of sedimentation time on the granulometric composition of suspended solids in the backwash water from biological activated carbon filters. *E3S Web of Conferences*, *44*, *00072*. EDP Sciences, DOI: 10.1051/e3sconf/20184400072 .

Korotta-Gamage, S.M. & Sathasivan, A. (2017). A review: Potential and challenges of biologically activated carbon to remove natural organic matter in drinking water purification process, *Chemosphere*, 167, pp. 120–138, DOI: 10.1016/j.chemosphere.2016.09.097.

Liao, X., Chen, C., Chang, C.-H., Wang, Z., Zhang, X. & Xie, S. (2012) Heterogeneity of microbial community structures inside the up-flow biological activated carbon (BAC) filters for the treatment of drinking water. *Biotechnology and Bioprocess Engineering*, 17, pp. 881–886, DOI: 10.1007/s12257-012-0127-x.

Lis, A., Pasoń, Ł. & Stępniak, L. (2016). Review of Methods Used to Indication of Biological Carbon Filters Activity. *Engineering and Protection of Environment*, 19, 3, pp. 413–425, DOI: 10.17512/ios.2016.3.11. (in Polish)

Mądrecka, B., Komorowska-Kaufman, M., Pruss, A. & Holc D. (2018). Metabolic activity tests in organic matter biodegradation studies in biologically active carbon filter beds. *Water Supply and Wastewater Disposal*, Politechnika Lubelska, 163–177.

Oliver, J.D. (2010) Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS Microbiology Review*, 34, 4, pp. 415–425, DOI: 10.1111/j.1574-6976.2009.00200.x.

Olszewska, M. & Łaniewska-Trokenheim, Ł. (2013) Fluorescence-based methods of cell staining in physiological state studies of bacteria. *Advancements of Microbiology*, *52*, *4*, pp. 409–418. (in Polish)

Papciak D., Kaleta J., Puszkarewicz A., Tchórzewska-Cieślak B. (2016). The use of biofiltration process to remove organic matter from groundwater. *Journal of Ecological Engineering*, *17*, 3, pp. 119–124, DOI: 10.12911/22998993/63481.

Pincus, D.H. (2013). Microbial identification using the bioMérieux Vitek 2 system, *Encyclo-pedia of Rapid Microbiological Methods*, PDA-DHI, p. 1–31. (https://store.pda.org/tableofcontents/ermm_v2_ch01.pdf)

Pruss, A. (2007): Contribution of Biofilm Thickness on Sand Filter Grains to Oxygen Uptake During Ammonia Nitrogen Removal. *Environmental Pollution Control*, 1, pp. 35–39. (in Polish)

Pruss, A., Maciołek, A. & Lasocka-Gomuła I. (2009). Effect of the Biological Activity of Carbon Filter Beds on Organic Matter Removal from Water. *Environmental Pollution Control*, *31*, pp. 31–34. (in Polish)

Sadowska J. & Grajek W. (2009). Analysis of physiological state of single bacterial cell using fluorescent staining methods. *Biotechnologia*, 4, pp. 102–114. (in Polish)

Seredyńska-Sobecka, B., Tomaszewska, M., Janus, M. & Morawski A. W. (2006). Biological activation of carbon filters. *Water Research*, 40, 2, pp. 355–363, DOI: 10.1016/j.watres.2005.11.014.

Simpson D. R. (2008). Biofilm processes in biologically active carbon water purification, *Water Research*, 42, 12, pp. 2839–2848, DOI: 10.1016/j.watres.2008.02.025.

Smith, A.C. & Hussey M.A. (2016) Gram Stain Protocols, *American Society for Microbiology*, pp. 1–9. (https://asm.org/getattachment/5c95a063-326b-4b2f-98ce-001de9a5ece3/gram-stain-protocol-2886.pdf)

Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., Wert, E.C. & Yoon, Y. (2007). Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. *Desalination*, 202, 1–3, pp. 156–181, DOI: 10.1016/j.desal.2005.12.052.

Standard Methods for the Examination of Water and Wastewater, 23'rd Edition, APHA, 2017 Washinghton.

Szeląg-Wasielewska, E., Joniak, T., Michałkiewicz, M., Dysarz, T. & Mądrecka, B. (2009) Bacterioplankton of the Warta River in relation to physicochemical parameters and flow rate. *Ecohydrology & Hydrobiology*, 9, 2–4, pp. 225–236, DOI: 10.2478/v10104-010-0008-x.

Szuster-Janiaczyk A. (2016). The Microbiological Evaluation of Deposits Come from Water Network on the Example of Selected Water Supply System. *Annual Set The Environment Protection*, 18, 2, pp. 815–827. (in Polish)

van der Kooij, D. & van der Wielen, P.W.J.J. (2014). Microbial Growth in Drinking-Water Supplies. Problems, Causes, Control and Research Needs, IWA Publishing, UK.

Van Nevel, S., Koetzsch, S., Proctor, C.R., Besmer, M.D., Prest, E.I., Vrouwenvelder, J.S., Knezev, A., Boon, N. & Hammes F. (2017). Flow cytometric bacterial cell counts challenge conventional heterotrophic plate counts for routine microbiological drinking water monitoring. *Water Research*, 113, pp. 191–206, DOI: 10.1016/j.watres.2017.01.065.

Wagner, M., Amann, R., Lemmer, H. & Schleifer, K. (1993). Probing activated sludge with oligonucleotides specific for Proteobacteria: inadequacy of culture-dependent methods for describing microbial community structure. *Applied and Environmental Microbiology*, 59, 5, pp. 1520–1525, DOI: 10.1128/AEM.59.5.1520-1525.1993.

WHO (2003). Expert consensus. In: Bartram J., Cotruvo J.A., Exner M., Fricker C.R., Glasmacher A. (Eds.) *Heterotrophic plate counts and drinking-water safety-the significance of HPCs for Water quality and human health*. IWA Publishing on behalf of the World Health Organisation, London.

Zamule, S.M., Dupre, C.E., Mendola, M.L., Widmer, J., Shebert, J.A., Roote, C.E. & Das P. (2021). Bioremediation potential of select bacterial species for the neonicotinoid insecticides, thiamethoxam and imidacloprid. *Ecotoxicology and Environmental Safety* 209, 111814; DOI: 10.1016/j.ecoenv.2020.111814.

Zhang, S., Gitungo, S.W., Axe, L., Raczko, R.F. & Dyksen, J.E. (2017). Biologically active filters – an advanced water treatment process for contaminants of emerging concern. *Water Research*, 114, pp. 31–41, DOI: 10.1016/j.watres.2017.02.014.

Ziglio, G., Andreottola, G., Barbesti, S., Boschetti, G., Bruni, L., Foladori, P. & Villa, R. (2002). Assessment of activated sludge viability with flow cytometry. *Water Research*, 36, 2, pp. 460–468, DOI: 10.1016/s0043-1354(01)00228-7.