Biostability of Tap Water—A Qualitative Analysis of Health Risk in the Example of Groundwater Treatment (Semi-Technical Scale)

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Abstract: This article presents results of research which aimed to assess the impact of biofiltration processing on the biological stability of water. Effectiveness of biogenic substances removal (C, N, P) and bacteriological quality of water after the biofiltration process were discussed. The research was carried out on a semi-technical scale on natural underground water rich in organic compounds. A filter with a biologically active carbon (BAC) bed was used for the research. Despite the low water temperature of between 9–12 °C, there was a high efficiency of organic matter removal—33–70%. The number of mesophilic and psychrophilic bacteria in the water before and after the biofiltration process was comparable (0–23 CFU/mL) and met the requirements for drinking water. No E. coli was detected in the water samples. The biological material washed out of the filter bed did not cause deterioration of water quality which proved that the operating parameters of the biofilters were properly chosen, i.e., contact time of 30 min, filtration speed up to 3 m/h. Reduction of the content of nutrients in the treated water limits the risk of microbial growth and thus the emergence of biological growth in the distribution system.

Keywords: bacteriological contamination of water; biofiltration; biological stability; water treatment

1. Introduction

The combination of unit processes used in water treatment technological systems should ensure not only removal of pollutants present in normative values but also guarantee water quality that reduces the risk of secondary contamination during transport to the recipient [1].

It is believed that the main cause of changes in the quality composition of water during its transport is, apart from the technical condition of the network, water instability. The result of instability is the intensification of corrosive processes through the precipitation and dissolution of accumulated deposits and the formation of biofilms [2,3]. All the factors enabling the creation and development of biofilms in water distribution systems are not presently sufficiently known [4]. A water that does not contain microorganisms and does not sustain their development in the water supply network is considered to be biologically stable. The striving to achieve the biological stability of water is associated with the need to ensure an extremely low content of food substrates, especially biodegradable organic carbon (BDOC), easily digestible nitrogen, and phosphorus [5–9].
The unsatisfactory efficiency of biogens removal has led to the need to expand conventional technological systems with a biofiltration process in which contaminants are removed by sorption, assimilation, and biodegradation [10–12].

Biofiltration makes it possible to eliminate the basic nutrients that ensure the growth of microorganisms and precursors of disinfection by-products (this is demonstrated by the decrease in UV absorbance) [13–15].

However, it remains doubtful how the efficiency of the biofiltration process will change after exhausting the adsorptive capacity of activated carbon. Will the water after the biofiltration process still be safe in sanitary terms and how will it affect the stability of tap water?

The greatest concerns relate to the possibility of microorganisms or fragments of biofilms, formed on activated carbon seeds which fill the biofilter, getting into the treated water. The fact that the final disinfection process does not completely eliminate bacteria, viruses, and fungi present in the treated water further intensifies these fears [16,17].

The aim of the research was to assess the impact of the biofiltration process on the microbiological quality of water and the effectiveness of biogenic substances removal which determine the biological stability of tap water.

2. Materials and Methods

2.1. Characteristics of Drawn Water (Raw)

The Water Treatment Plant (WTP) is supplied from an unconfined quaternary aquifer with a depth of about 15 m bgl using 27 wells. The physicochemical quality of the water being captured is presented in Table 1.

| Indicator                  | Unit       | Range of Changes |
|----------------------------|------------|------------------|
| Total Organic carbon (TOC) | g O₂/m³    | 11.0–14.5        |
| Permanganate value         | g O₂/m³    | 11.0–18.1        |
| Turbidity                  | NTU        | 8.0–14.0         |
| Colour                     | g Pt/m³    | 40–100           |
| Ammonia nitrogen           | g NH₄⁺/m³  | 1.20–1.98        |
| pH                         | -          | 6.4–7.0          |
| Temperature                | ºC         | 10.8–12.1        |
| Hardness                   | g CaCO₃/m³ | 200–470          |
| Sulfate                    | g SO₄²⁻/m³ | 60–240           |
| Conductivity               | mS/cm      | 430–1016         |
| Alkalinity                 | val/m³     | 2.5–4.5          |

2.2. Technology System for Water Treatment

The drawn water is directed to a collective well (stoppage time of 2–4 h, depending on the current water production). The water is then directed to a cascade of oxygenation. A dispensing potassium permanganate chemical-oxidant with a dose of 2.1–2.4 mg/L and a PAX-18 coagulant in the amount of 120–140 mg/L is situated just below the cascade (11.0–12.6 mg Al/L). Vertical coagulation–sedimentation chambers with a contact time of 6–8 h are the next stage of the water treatment. Lime milk is dosed (about 10.0 mg/L) to the water in the chambers, which is directed to horizontal settler raising the pH. The retention time in the settlers is several hours. The next step involves filtration of water at a speed of 1.5–3 m/h and the final disinfection with sodium hypochlorite (Figure 1).
Figure 1. Technological flowchart for the Water Treatment Plant.

The filter used was a biologically active deposit of activated carbon with the parameters contained in Table 2.

Table 2. Parameters of the biofilter model [18].

| Operating Parameters          | Value | Properties of the Filter Material | Value         |
|------------------------------|-------|-----------------------------------|---------------|
| Height of the carbon bed, m  | 1.12  | Granulation, mm                   | 1–4           |
| Diameter, m                  | 0.055 | Specific surface, m²/g            | 950–1050      |
| Filtration velocity, m/h     | 1.5–2.0| The iodine value, mg/g             | 998           |
| Contact time, h              | 0.5   | pH aqueous extract                | 11            |

2.3. Research Procedure

The research was carried out over a period of one calendar year. The list of controlled water parameters and analysis procedures is presented in Table 3. The obtained results were subjected to statistical analysis with the use of STATISTICA12 software.

2.4. Evaluation of Work of Model Filter-Test EMS

The development of activity within biosorption beds, and the observations regarding the relationships between sorption and biodecomposition processes, were recorded based on the Eberhardt, Madsen, Sontheimer (EMS) test [19–21]. The EMS test is based on the value of the indicator, described by the relation between the change in COD or permanganate index and the loss of dissolved oxygen, taking place during filtration.

\[ S = \frac{\Delta \text{COD}}{\Delta \text{O}_2} \]  

where:

\( \Delta \text{COD} \) — loss of COD (with \( \text{K}_2\text{Cr}_2\text{O}_7 \) or with \( \text{KMnO}_4 \))

\( \Delta \text{O}_2 \) — loss of dissolved oxygen

When:

- \( S = 1 \) adsorption and biodecomposition happen with identical intensiveness,
• S > 1 adsorption dominates,
• S < 1 biodecomposition dominates,
• S = 0, ΔCOD = 0, ΔO₂ > 0 sorption and biodecomposition processes stopped
• S undetermined, ΔCOD > 0, ΔO₂ = 0 sorption present, biodecomposition absent ΔCOD = 0, ΔO₂ = 0 sorption and biodecomposition absent.

The presence of the biofilm was confirmed by observation, using a light microscope and an electron scanning microscope JOEL SEM 5500 LV (JEOL Ltd., Tokyo, Japan).

Table 3. Summary of analytical methods for laboratory experiments [22].

| Parameters                          | Analytical Method/Standard                                                                 |
|-------------------------------------|-------------------------------------------------------------------------------------------|
| Total organic carbon (TOC)          | TOC analyzer Sievers 5310 C (SUEZ, Boulder, CO, USA)                                     |
| Permanganate value                  | The permanganate method                                                                   |
| UV absorbance                       | Spectrophotometric method                                                                |
| Inorganic nitrogen content (N-NH₄⁺ + N-NO₂⁻ + N-NO₃⁻) | N-NH₄⁺: direct nesslerization method using Merck spectrophotometer                         |
|                                    | N-NO₂⁻: colorimetric method by Nitrite Test Merck 1.14408                                 |
|                                    | N-NO₃⁻: spectrophotometric method with sodium salicylate                                  |
| Inorganic phosphorus content        | Spectrophotometric method with ammonium molybdate using Merck spectrophotometer          |
| Dissolved oxygen (DO)               | Electrochemical method using a Hach-Lange oxygen probe                                    |
| The total number of bacteria at 37 °C after 24 h (mesophilic bacteria) | Traditional culture method using A Agar from BTL Ltd.                                    |
| The total number of bacteria at 22 °C after 72 h (psychrophilic bacteria) | Membrane filtration procedure using Endo agar                                           |
| Escherichia coli bacteria            |                                                                                           |

3. Results

Expanding a conventional treatment system with a biofiltration process on granulated active carbon resulted in an improvement of physicochemical parameters (Figure 2, Figure 3 and Figure 9) and did not cause the deterioration of the treated water’s microbiological parameters (Figures 7 and 8).

The average monthly values of total organic carbon in the water feeding the carbon filter ranged from 8.13 to 11.61 mg C/dm³, whereas, after the biofiltration process, the values ranged from 3.47 to 6.31 mg C/dm³ (Figure 2). The highest efficiency of removing organic compounds, by 70%, was observed in February. From April to November, the effectiveness of total organic carbon (TOC) removal was relatively stable at 33%.

The average efficiency of absorbance reduction after the biofiltration process ranged from 4% to 72%, and the lowest obtained value was 3.40 m⁻¹ (Figure 2). The analysis of obtained test results showed a strong relationship between the concentration of TOC and UV absorbance, for which the Pearson correlation coefficient reached a value of R = 0.77. The measurement of the total organic carbon content at many water treatment plants is often replaced by the ultraviolet absorbance test.

The concentration of organic matter expressed by oxidation fluctuated around the recommended value (5 mg O₂/dm³), while the introduction of a biofiltration process allowed lowering of the average monthly values of this parameter by a factor of two (Figure 3).

The increase in the efficiency of organic matter removal in the filtration process through a bed of activated carbon was mainly caused by the process of biodegradation occurring with the participation of microorganisms developing on the surface of grains [19]. The dominant biosorption process was demonstrated by the reduction of oxidation and oxygen depletion (Figures 3 and 4).
The nature of the carbon bed’s work and its biological activity was determined using the Eberhardta, Madsena, and Sointheimera (EMS test). The average values of the S index obtained for the one-year study are shown in Figure 4.

The EMS test confirmed that the biodegradation process dominates on the surface of the activated carbon. S index values were in the range from 0.26 to 0.8. The exception was in the months of March and April, during which the increase of S index was above 1, indicating the dominance of the sorption process over biodegradation.

The presence of biofilm on the surface of activated carbon is confirmed by images from light (Figure 5) and scanning microscopes (Figure 6).

The average number of mesophilic and psychrophilic bacteria in the water before and after the process was at a comparable level, i.e., 0–18 CFU/mL in the case of mesophilic and 0–23 CFU/mL—psychrophilic (Figure 7). The presence of *E. coli* was not detected in the water samples after the biofiltration process during the entire test period. The presence of *E. coli* in an amount of 1–3 CFU/mL constitutes low, 4–6 CFU/mL medium, and 7–9 CFU/mL a high health risk [23].

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**Figure 2.** Average monthly values of total organic carbon and UV absorbance in water before and after the biofiltration process.

**Figure 3.** Average monthly values of permanganate value and dissolved oxygen in water before and after the biofiltration process.
Figure 4. Average monthly changes in S indicator after biofiltration process.

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

S > 1 adsorption dominates
S < 1 biodegradation dominates,
S = 1 adsorption and biodegradation occurs with the same intensity

Figure 5. Edge of granular activated carbon grain covered with biological membrane (photos from a light microscope).

Figure 6. Biological membrane formed on granular activated carbon ((A): before biosorption, (B): after biosorption)—photos from a scanning microscope.
Introduction of the biofiltration process allowed obtaining of water that meets the microbiological requirements as defined by Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. In addition, the quality of treated water corresponded to the requirements for bottled water (bottle sales), as the number of mesophilic and psychrophilic bacteria did not exceed the normative values of 20 and 100 CFU/mL, respectively (Figure 7) [24].

The statistical analysis of the microbiological research results (Figure 8) fully confirms that a properly conducted biofiltration process is safe. The biological material washed out of the filter bed will not affect the sanitary condition of water if the biofilter parameters are optimal for the biodegradation process, i.e., contact time (30 min), filtration speed (1–3 m/h).

Figure 9 compares the physicochemical quality of water before and after the biofiltration process on granulated activated carbon. Reducing the content of nutrients in water limits the risk of loss of biological stability of water entering the distribution system. The threshold values of parameters limiting secondary development of microorganisms in distribution systems should be lower than 0.25 g C/m$^3$ BDOC, 0.2 g N$_{norg}$/m$^3$ and 0.03 g PO$_4^{3-}$/m$^3$ [25].

![Figure 7. Total number of mesophilic and psychrophilic bacteria in water before and after the biofiltration process.](image-url)
For the whole of the research period, the average content of inorganic nitrogen in water reaching the biofilter was 0.76 g N\textsubscript{norg}/m\textsuperscript{3}, whereas after filtration through the active carbon bed it was 0.62 g N\textsubscript{norg}/m\textsuperscript{3}. The value recommended for maintaining biostability—0.2 g N\textsubscript{norg}/m\textsuperscript{3}—was not obtained (Figure 10). The content of phosphate ions varied in the range of 0–0.0035 g PO\textsubscript{4}\textsuperscript{3–}/m\textsuperscript{3} and met the requirements necessary to maintain water biostability (0.03 gPO\textsubscript{4}\textsuperscript{3–}/m\textsuperscript{3}) (Figure 10).

Analyzing the three-level scale of risk, i.e., tolerated, controlled, and non-acceptable risk, it was found that in a conventional treatment system, only 4.7% of the samples tested are in the tolerable risk zone, while the biofiltration process increases this value to 28.6%. The remaining samples correspond to the level of safety requiring control and reduction, in which there are reasons to maintain water biostability [14].

The comparison of median of the treated water’s physicochemical parameters in a conventional system and in the system extended by the biofiltration process indicates the justifiability of introducing biofiltration using granulated activated carbon. Reducing the content of nutrients in treated water reduces the risk of microbial growth and thus the emergence of biological growth in the distribution system.

![Figure 8](image1.png)
**Figure 8.** Statistical analysis of microbiological results obtained for water before and after biofiltration process on granular activated carbons.

![Figure 9](image2.png)
**Figure 9.** Statistical analysis of selected physicochemical results obtained for water before and after the biofiltration process on granular activated carbon deposits. Designations: (1) TOC (g C/m\textsuperscript{3}); (2) UV absorbance (UV\textsuperscript{im 254}); (3) Permanganate value (g O\textsubscript{2}/m\textsuperscript{3}); (4) Dissolved oxygen (g O\textsubscript{2}/m\textsuperscript{3}); (5) Total inorganic nitrogen (g N\textsubscript{norg}/m\textsuperscript{3}); (6) Total phosphorus (g PO\textsubscript{4}\textsubscript{3–}/m\textsuperscript{3}); (N = 44).
Before the biofiltration process

After the biofiltration process

Water temperature is an important parameter determining the activity of microorganisms. Moll et al. found that the removal efficiency of dissolved organic carbon at 20 °C was 24%, and at 5 °C the efficiency was reduced to 15% [34]. Emelko et al. observed similar differences in the efficiency of the biodegradation process—92% for 21–25 °C and 58% for 1–3 °C [35]. Selbes et al. found seasonal variability of biofiltration efficiency. The effectiveness of removing dissolved organic carbon was lower in cooler months (~5%) and higher in warmer months (~24%) [36]. Halle et al. also confirm that the supply water temperature affects the efficiency of biofiltration [37]. Low temperature not only reduces the rate of metabolism of the substrate but also affects the structure and diversity of the biological membrane [34,38]. Hallam determined that the microbial activity at 7 °C is 50% lower than at 17 °C [3].

In the analyzed case, the problem concerned removal of organic matter from groundwater, which, unlike surface water, is characterized by low temperature variability throughout the year. The temperature could not constitute the main reason for reducing the activity of microorganisms because the highest efficiency of biofilters was observed in the winter months, i.e., December–March.

Literature indicates that the microbial activity correlates with the availability of nutrients, i.e., the content of carbon, nitrogen, and phosphorus in the water flowing into the biosorption field. The C:N:P ratio should be 100:10:1 and deficiency of any of the constituents limits the growth and development of microorganisms, thus interfering with the course of the biodegradation process [39,40]. Dhawan

Figure 10. Average monthly values of total inorganic nitrogen and total phosphorus in water before and after the biofiltration process.

4. Discussion

The effectiveness of removing organic pollutants on active carbon beds has been confirmed by numerous studies [15,19,26–29]. Conventional water treatment systems eliminate natural organic substances up to 40% [11,21,24], while systems extended by biofiltration, depending on the time of biofilter exploitation, achieve from 24–100% [15,19,30–32]. The effectiveness of organic matter removal in metabolic processes is lower (24–42%) than in the adsorption process (70–100%), but the effectiveness of biofilter operation may exceed 10 years [10,33].

The research conducted for the first operational period of the biofilter [19] showed that the combination of sorption processes with biodegradation until exhaustion of the capacity of the carbon bed allowed for 100% removal of organic matter. Creation of biofilm before exhaustion of the sorption capacity of carbon allowed extending of the filtration cycle but the effectiveness of removing TOC decreased to 70% [19].

The results of research presented in this paper indicate that after a year of using a biofilter the efficiency of organic matter removal decreased by 50%. The observed decrease in the effectiveness of the biofilter could be caused by the depletion of the sorption capacity of carbon and the decrease in the activity of microorganisms inhabiting the carbon bed.

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et al. controlled the effectiveness of three biofilters in which the treated water differed in terms of nutrient content as follows: 54:24:1, 100:10:1, and 25:10:1. The Dissolved Organic Carbon (DOC) removal efficiency was 23.5%, 28.6%, and 33.5% respectively [40]. In the case of waters containing natural organic matter and inorganic nitrogen, phosphate ions are essential [41,42]. Their insufficient content inhibits the growth of microorganisms to a much greater extent than is the case for other biogens [6,43]. Phosphorus concentrations below 0.005 mg/dm$^3$ inhibit the transport of phosphorus to bacterial cells [44].

The water flowing into the biofilter was characterized by a low phosphorus content (0–0.0035 g PO$_4^{3-}$/m$^3$) which could have influenced the activity of microorganisms and the efficiency of the biofiltration process. The observed lack of changes in the content of phosphorus in water after biofiltration with the simultaneous removal of nitrogen (18%) and TOC (33%) could suggest the existence of another source of phosphorus. It is likely that the carbon that fills the biofilter has been chemically activated with phosphorus-containing compounds.

It should be noted that despite the unfavorable relationship between the content of biogenic substances (C:N:P, 28500:217:1), there has been a smooth transition of the sorption process into biodegradation. The only explanation for the effective operation of the biofilter in the initial period of exploitation could be a method of coal grains’ chemical activation. Perhaps supplementing the phosphorus content in an amount that would allow the optimal biogen ratio would help to increase the efficiency of natural organic matter removal. The decisive role of phosphorus should be kept in mind when water directed to a biofilter is subjected to coagulation, effectively eliminating phosphorus from the treated water.

The effectiveness of removing impurities in the BAC process also depended on the activity and type of microorganisms forming the biological membrane [33,45]. Studies on the biological activity of carbon bed samples were presented, among others, in the thesis [28,46–48]. Literature data show that the biomass concentration demonstrates vertical stratification and decreases with the depth of the bed, however [45,47,49–52], this is not the rule [53]. Differences in the composition and concentration of biomass depend on the availability of nutrients and the concentration of dissolved oxygen [54]. Less attention has been paid to the microbiological quality of the filtrate. In the works [55,56], small changes were shown between the quality of water directed to biofilters and flowing from the biofilter (the study was conducted for surface water treated with ozonation before biofiltration).

When analyzing the microbiological quality, it was found that the number of mesophiles and psychrophiles in water before and after biofiltration was comparable (0–23 CFU/mL). The biological material washed out of the filter bed did not cause deterioration of water quality which proved that the operating parameters of the biofilters were properly chosen, i.e., contact time (30 min), filtration speed (up to 3 m/h).

5. Conclusions

The biofiltration process improves the quality and biological stability of treated water, reducing the health risk and risks associated with threats to the technical infrastructure.

The number of mesophilic and psychrophilic bacteria in the water before and after biofiltration process was comparable (0–23 CFU/mL) and met the requirements for drinking water. No *E. coli* was detected in the water samples. The biological material washed out of the filter bed did not cause deterioration of the water quality which proved that the operating parameters of the biofilters were properly chosen.

The efficiency of TOC removal in the biofiltration process ranged from 70% to 33%. The reason for the decrease in the effectiveness of the process of organic matter removal in the second year of biofilter exploitation could be depletion of the adsorption capacity of the carbon bed and reduction of the activity of microorganisms colonizing the bed. Too low content of phosphorus in the water entering the biofilter could affect the efficiency of the biodegradation and assimilation process.
The values of the median of treated water’s physicochemical and bacteriological parameters in the system extended by the biofiltration process prove the justifiability of introducing this process into the technological system of water treatment.

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