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ORIGINAL ARTICLE

EVALUATION OF SUSCEPTIBILITY OF POLYMER AND RUBBER MATERIALS INTENDED INTO CONTACT WITH DRINKING WATER ON BIOFILM FORMATION

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ABSTRACT

Background. Plumbing materials in water distribution networks and indoor installations are constantly evolving. The application of new, more economical solutions with plastic materials eliminates the corrosion problems, however, do not fully protect the consumer against secondary microbial contamination of water intended for human consumption caused by the presence of a biofilm on the inner surface of materials applied. National Institute of Public Health - National Institute of Hygiene conducts research aimed at a comprehensive assessment of this type of materials, resulting their further marketing authorization in Poland.

Objectives. Evaluation and comparison of polymer and rubber materials intended to contact with water for the susceptibility to biofilm formation.

Materials and Methods. Plastic materials (polyethylene, polypropylene, polyvinyl chloride) and rubber compounds (EPDM, NBR), from different manufacturers were evaluated. The study was carried out on 37 samples, which were divided into groups according to the material of which they were made. The testing was conducted according to the method based on conditions of dynamic flow of tap water. The level of bioluminescence in swabs taken from the surface of the tested materials was investigated with a luminometer.

Results. Evaluation of plastic materials does not show major objections in terms of hygienic assessment. All materials met the evaluation criteria established for methodology used. In case of rubber compounds, a substantial part clearly exceeded the limit values, which resulted in their negative assessment and elimination of these materials from domestic market.

Conclusions: High susceptibility to the formation of biofilm in the group of products made of rubber compounds has been demonstrated. Examined plastic materials, except for several cases, do not revealed susceptibility to biofilm formation, but application of plastics for distribution of water intended for human consumption does not fully protect water from secondary, microbiological contamination. Complete verification of plumbing materials including biofilm formation test before their introduction into the domestic market should be continued.

Key words: *biofilm, plumbing materials, hygienic assessment, drinking water*

STRESZCZENIE

Wprowadzenie. Materiały wykorzystywane w sieciach wodociągowych oraz instalacjach wewnątrz budynków ulegają ciągłym zmianom. Wprowadzenie nowych bardziej ekonomicznych rozwiązań z zastosowaniem materiałów z tworzyw sztucznych eliminuje problemy związane z korozją, jednak nie zabezpiecza konsumenta przed wtórnym mikrobiologicznym zanieczyszczeniem wody przeznaczonej do spożycia powodowanym występowaniem biofilmu na wewnętrznej powierzchni rur i przewodów instalacyjnych. W Narodowym Instytucie Zdrowia Publicznego - Państwowym Zakładzie Higieny, prowadzone są badania mające na celu kompleksową ocenę tego typu materiałów, czego efektem jest wydawanie Atestów Higienicznych i dopuszczenie materiałów do obrotu na krajowym rynku.

Cel. Celem badań było porównanie i ocena materiałów z tworzyw sztucznych i gumy przeznaczonych do kontaktu z wodą do spożycia w zakresie ich podatności na tworzenie biofilmu.

Materiał i metody. Ocenie poddano materiały z różnych tworzyw sztucznych (polietylen, polipropylen, polichlorek winylu) oraz mieszanki gumowe pochodzące od różnych producentów. Badania wykonano dla 37 próbek, które zostały podzielone na grupy w zależności od rodzaju materiału z jakiego zostały wykonane. Badania prowadzone były zgodnie z metodyką własną, w dynamicznych warunkach przepływu wody, z wykorzystaniem urządzeń przepływowych (UPE). Za pomocą luminometru oznaczano poziom bioluminescencji w wymazach pobranych z powierzchni testowanych materiałów.

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Wyniki. Ocena zbadanych materiałów z tworzyw sztucznych nie budziła większych zastrzeżeń pod względem higienicznym. Wszystkie materiały spełniały kryteria oceny określone w stosowanej metodyce badawczej, przy czym w kilku przypadkach oznaczone wartości były bliskie dopuszczalnego limitu. W przypadku mieszanek gumowych, w znacznej części stwierdzono wyraźne przekroczenia dopuszczalnych wartości, co skutkowało negatywną ich oceną oraz eliminacją tych materiałów z obrotu na krajowym rynku i możliwością ich wykorzystywania w kontakcie z wodą do picia.
Wnioski. Wykazano znaczną podatność zbadanych produktów wykonanych z mieszanek gumowych na tworzenie się biofilmu. Materiały tworzywowe jak polietylen, polichlorek winylu i polipropylen, w znacznej większości nie wykazywały podatności na tworzenie się biofilmu, jednak ich zastosowanie do dystrybucji wody przeznaczonej do spożycia przez człowieka nie zabezpiecza jej w pełni przed wtórnym, mikrobiologicznym zanieczyszczeniem. W celu pełnej weryfikacji materiałów, przed ich wprowadzeniem na krajowy rynek, niezbędna jest dalsza ich ocena, z uwzględnieniem ich podatności na tworzenie się biofilmu.

Słowa kluczowe: *biofilm, materiały instalacyjne, ocena higieniczna, woda przeznaczona do spożycia*

INTRODUCTION

To the end of the 90's of the last century the water supply systems in Poland were made of traditional materials like cast iron. They accounted for over 50% of the network and their technical condition due to the long lifetime (over 50 years) was of low quality [6]. However, these installations underwent successive repairs and upgrades, which entailed also change of materials used for this purpose. Since the beginning of the twenty-first century, it was observed a significant part of different material solutions like polyethylene (PE) and polyvinyl chloride (PVC) as well as an increasing use of a new generation of iron, so-called ductile iron [15, 16, 18]. Currently, a significant share in the materials structure have pipes made of polymer materials (PE, PVC and others), which in some cases account for more than a half of the length of water supply networks. Generally, it can be said, that the water supply systems are built mainly of pipes made of cast iron, steel protected against corrosion usually by zinc, cementitious coating, PVC and PE. The pipes made of these materials account for about 90% of the network length [14, 17]. In fact, it does not differ much from the standards in other European Union countries, as well as in the United States [25]. Plastics intended for use in the construction industry, especially those that can be applied in contact with drinking water must meet a number of strict requirements, not only technical like strength, flexibility and corrosion resistance, but primarily hygienic requirements, which apply to both, the physicochemical and microbiological parameters. All over the world the research is carried out focused on the acceptance of different products/materials contacting with water intended for human consumption. These tests include so-called migration tests, performed to proof that the product/material has no negative influence on the quality of water contacting with it, and microbiological tests, including biofilm formation test. The hygienic assessments of products intended to contact with drinking water, are performed, among others, in the research centers in Germany (Gelsenkirchen Institute), France – Pasteur Institute (ACS, AFNOR), Great Britain (WRAS), the Netherlands (KIWA), United States (NSF) and in many other countries, including Poland. Here in the National Institute of Public Health – National Institute of Hygiene (NIPH-NIH) hygienic assessment of materials is conducted during procedure of Hygienic Certificate issuing [3, 7, 12, 27, 30]. These centers conduct physicochemical and microbiological research, but only some of them carry out tests on microbial growth (biofilm formation test) for plastic and rubber products. Increasing number of metallic materials is replaced by polymer materials because of their high chemical resistance, good mechanical properties, which can be improved through the addition of modifiers like chalk, carbon black, chopped glass and elastomers. The use of polymer materials eliminates corrosion, known from occurrence in traditional materials (like cast iron, steel, concrete), provides channels tightness, even in critical situations (deflection instead of cracking) and guarantees proper attention to economy of the solutions. However, an attention should be payed to possible interactions that occur between the microorganisms present ~~ce~~ in tap water, and the material from which the network is made. In water environment, even inside the pipes and lines, which supply drinking water to consumer, there are two main forms of the presence of microorganisms. They can float freely in unbound form or bound with molecules of organic or inorganic matter suspended in water (planktonic form) or they can form complex agglomerates permanently colonizing the inner surfaces of the pipes - biofilm. Both these forms are not mutually exclusive, but microorganisms forming biological membranes are more frequently observed [22]. Biofilm, also defined as a biological membrane, is a three-dimensional, spatially complex structure, arising at the phases border, including different kinds of organic and inorganic surfaces contacting with water. The composition of the biofilm can vary. It can be a monoculture or a cluster of bacteria, very diverse morphologically and physiologically [4, 21]. Biofilm formed in plumbing installation is a very common phenomenon. Once produced, becomes very difficult to remove and can cause many problems of technological importance by intensifying processes of biological corrosion and causing significant hydraulic losses inside the network and, above all, it may cause the health problems to consumer [13, 26]. Biofilm creates very favorable living conditions for microorganisms, provides them with a greater availability of nutrients [5, 23] and enables long-lasting and very stable settlement of diverse solid substrates, including construction materials contacting with water. Their presence has an impact on the quality of water delivered to the consumer. Frequent detachment of fragments of the mature biofilm inside water network causes ejection of a large number of bacteria, including pathogens and potential human pathogens such as *Legionella pneumophila*, *Pseudomonas* sp., *Aeromonas* sp., *Campylobacter* sp., *Escherichia coli*, *Salmonella* sp., *Shigella* sp. as well as microscopic fungi [9, 10, 19, 29]. During exploitation of the water network, a permanent growth of the biofilm is observed, wherein the biomass of bacteria can reach 95% and only 5% of bacteria is present in the water in the form of so-called phytoplankton [20].

Therefore, it is important to choose the appropriate materials, which are used to build a water supply system at the first stage of its design. It enables avoiding the subsequent operational problems associated with the metabolic activity of microorganisms. Due to the extension of the hygienic certification procedure conducted in NIPH – NIH on biological laboratory tests allowing to perform complete assessment of the materials intended to contact with drinking water. It is possible to verify the quality of certified materials and to eliminate those, that due to the high susceptibility to biofilm formation, can cause secondary microbial contamination of water supplied to the consumer.

The aim of this study was the evaluation and comparison between some polymer materials and elements made of rubber intended to contact with drinking water on their susceptibility to biofilm formation.

MATERIALS AND METHODS

The study was carried out on 37 samples, which were divided into several groups according to the material of which they were made, enabled the general characteristics of each group.

Tested materials

During the research the following polymer materials were used: polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) and rubber compounds (EPDM, NBR and others). Polymers as main compounds of plastic materials are safe for human health. However, the remains of unreacted monomer and modified additives can be volatile and toxic. Modifiers usually in the form of low molecular weight compounds can penetrate from the plastic to the water and deteriorate its quality, thus providing nutritional compounds for microbes in tap water. Among the huge amount of rubber compounds the largest part of the sanitary products are those made of EPDM and NBR. They are characterized by a high chemical resistance including a resistance to atmospheric agents. They meet the specific requirements associated with the flow of hot water (high temperature resistance), especially in the case of EPDM and low deformation, which translates into prolonged use. Unfortunately, materials like rubber compounds and polymers contacting with drinking water may cause the secondary pollution both, chemical and microbiological.

All tests were performed using two control reference materials. Positive control (susceptible to biofilm formation) was glass plate coated with paraffin wax layer. Negative control (unsusceptible to biofilm formation) was plate made of stainless steel.

Continuous flow reactor

Specific reactors - UPE (Polish specific name) supplied from cold tap water were used in this investigation (Figure 1). The cylindrical body of UPE (inside diameter was 150 mm, high was 550 mm) was made of high quality stainless steel with Teflon seal. On top of the reactor was a removable cover with a venting valve. Water inflow tube was made of Teflon and water pressure was regulated by a ball valve. Inside the reactor, at the bottom, a conical diffuser with two partitions was located. A special sample stand made of stainless steel was placed in vertical position inside the cylinder. The water outflow with water meter was located on the side of the UPE about 50 mm below the top. The water flow proceeded from the bottom to the top of reactor so that the reactor could be filled with water evenly and flow direction was protected against the mixing of inlet and outlet water.

The testing method applied in the Department of Environmental Hygiene Laboratory of NIH-NIPH involves the measure of bioluminescence level in swabs taken from the surface of material contacting with drinking water by using luminometer. Exposition of tested material lasts from eight (polymers) to ten weeks (rubber) and is performed inside the continuous flow reactor (UPE) in conditions of dynamic water flow. The crucial element of the testing method is the fact, that all living cells include the universal chemical compound - adenosine triphosphate (ATP) which functions as a carrier of free energy. This energy is used in most of the life processes requiring the energy input. During biochemical reaction of enzymatic decomposition of ATP energy is emitted in form of light (bioluminescence). The measure of this energy enables indirect assessment of ATP concentration in swab sample taken from the surface of tested material.

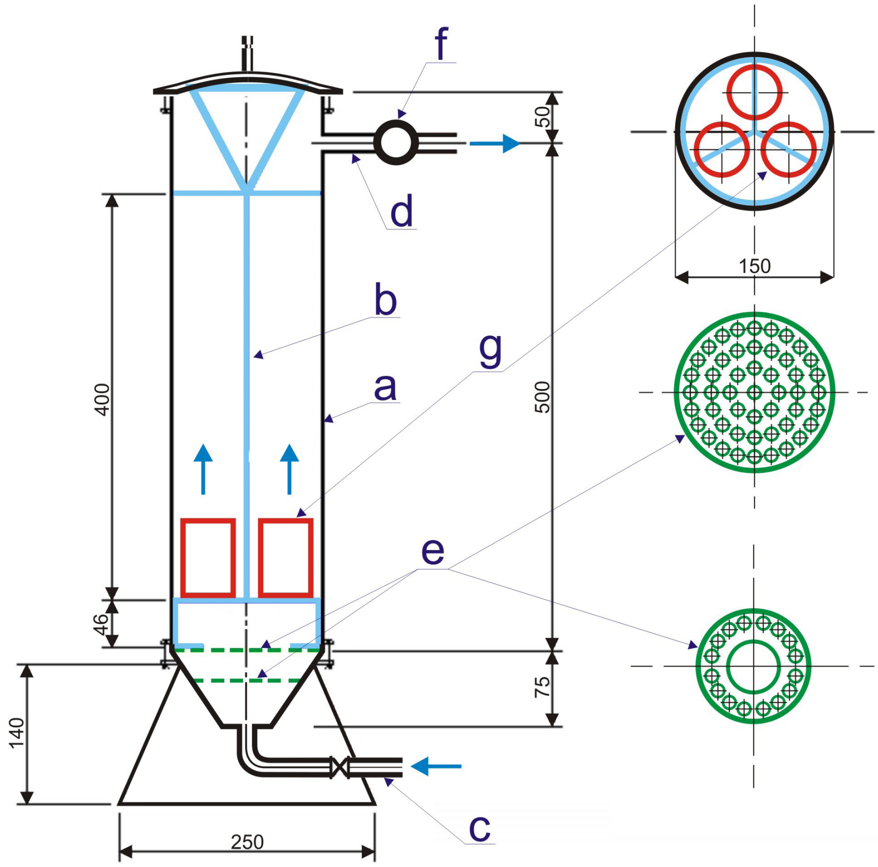

Figure 1. Continuous flowing reactor's diagram. a – steel cylinder, b – samples stand, c – water inflow, d – water outflow, e – diffuser, f – water meter, g – sample of material intended to contact with water.

HY-LiTE® (MERCK) test systems to *in vivo* application were used to bioluminescence level measures. The test system consists of hygiene swabs free of ATP and the "pens" with proper dose of liquid reagents to dilution, buffering and neutralization of the sample, as well as lyophilisate of reaction complex luciferin-luciferase. This test system was adapted to operate with luminometer HY-LiTE 2® (MERCK). The sensitivity of applied tests was 1,4 x 10^{-14} mols ATP.

RESULTS

The tests results on biofilm formation process for various types of materials intended for contact with drinking water from different producers were presented in Tables 1, 2 and 3.

Ten different products made of polyvinyl chloride intended to contact with drinking water were tested and evaluated. The results obtained for all PVC samples were similar (Table 1). In each case values expressed in RLU/cm^2 did not exceed the acceptable limit determined pursuant to ten-time values observed on the negative control surface (10 x K-). Sample number 5 characterized the highest bioluminescence values, however the values were below acceptable limit during full time of investigation. In all cases presented above, examined materials obtained positive test assessment, what confirmed their proper quality and resulted in issuing of the Hygienic Certificate and further marketing authorization in Poland.

Results for tested seven different products made of polypropylene from different producers are presented in Table 2. The bioluminescence level on the surface of six of them was low (<500 RLU/cm^2). In case of one material (sample 2) the bioluminescence level, measured in the measuring period, exceeded slightly 2000 RLU/cm^2, however the negative control sample, tested in parallel, showed results 253 RLU/cm^2. Thus, working on the assumption of the assessment criteria for the method (10 x K-), enabled to positive assessment of this material sample made of polypropylene. All samples made of polypropylene obtained positive test assessment, what confirmed their proper quality and resulted in issuing of the Hygienic Certificate and further marketing authorization for direct contact with water intended for human consumption.

Ten different products intended to contact with drinking water and made of polyethylene from different producers were tested. The results for all samples presented above were similar (Table 3). In case of eight of them, during eight week examined period, the bioluminescence was observed on the level very close to negative control, so in the range from approximately 100 RLU/cm^2 to 300 RLU/cm^2. In three cases (samples no. 3, 4 and 10) the values obtained were higher and amounted above 1000 RLU/cm^2. They still did not exceed the unacceptable value limit.

All examined polyethylene materials obtained positive test assessment, what confirmed their proper quality and resulted in issuing of the Hygienic Certificate and further marketing authorization in Poland.

Table 1. Bioluminescence on the surface of different polyvinyl chloride (PVC) materials expressed in RLU*/cm2

PVC	Subsequent weeks of the study								
	0	I	II	III	IV	V	VI	VII	VIII
sample 1 limit value**	36 170	358 750	721 1100	900 1350	800 1410	870 1630	950 1540	970 1490	1090 1510
sample 2 limit value	18 140	29 650	39 1050	29 2250	62 920	175 1250	457 1020	321 950	125 820
sample 3 limit value	29 130	93 1450	65 770	85 1880	142 2010	162 2070	150 1540	127 1120	110 790
sample 4 limit value	20 310	159 1100	107 1620	187 1160	225 720	188 1210	153 1420	137 1350	73 1470
sample 5 limit value	21 300	155 1100	171 810	166 1400	266 610	411 750	633 1000	670 870	600 900
sample 6 limit value	19 370	56 660	89 1210	74 2100	151 2580	234 3090	287 2740	211 3660	178 3530
sample 7 limit value	19 140	67 800	98 1250	165 1600	170 1400	260 850	210 910	180 1110	160 780
sample 8 limit value	11 90	45 330	90 560	125 800	160 1000	230 1200	250 1450	240 1600	260 1700
sample 9 limit value	11 120	56 450	94 800	144 900	187 1320	156 1600	210 1650	183 1910	162 1800
sample 10 limit value	9 120	40 450	66 800	97 900	110 1320	121 1600	106 1650	120 1910	115 1800

* - Relative Light Units
** - acceptable limit of RLU/cm2 determined pursuant to ten-time values observed on the negative control surface (10 x K-)

Table 2. Bioluminescence on the surface of different polypropylene materials expressed in RLU/cm2

PP	Subsequent weeks of the study								
	0	I	II	III	IV	V	VI	VII	VIII
sample 1 limit value	29 370	95 660	134 1210	195 2100	254 2580	312 2090	378 2340	472 2660	456 2530
sample 2 limit value	30 370	150 660	317 1210	1000 2100	1356 2580	1389 2090	2389 2340	2100 2660	2433 2530
sample 3 limit value	37 370	156 660	140 1210	143 2100	158 2580	165 3090	156 2740	147 3660	166 3530
sample 4 limit value	29 370	98 660	123 1210	167 2100	197 1950	211 2210	356 2740	287 3000	311 2470
sample 5 limit value	25 140	25 650	26 1050	57 2250	248 920	48 1250	350 1020	190 950	160 820
sample 6 limit value	37 310	170 1100	110 1620	120 1160	138 720	145 1210	126 1420	147 1350	136 1470
sample 7 limit value	9 120	64 450	159 800	264 900	350 1320	445 1600	474 1650	450 1910	463 1800

Table 3. Bioluminescence on the surface of different polyethylene materials expressed in RLU/cm²

PE	Subsequent weeks of the study								
	0	I	II	III	IV	V	VI	VII	VIII
sample 1 limit value	37 300	180 1110	130 980	140 1330	138 810	175 750	156 930	127 960	146 740
sample 2 limit value	36 230	106 560	182 890	274 1740	286 1470	311 1620	246 1850	198 1770	233 1810
sample 3 limit value	30 370	130 660	217 1210	736 2100	1416 2580	1311 3090	2367 2740	1976 3660	2133 3530
sample 4 limit value	21 150	97 350	196 220	311 900	560 1640	710 1870	900 1680	732 2100	1162 1640
sample 5 limit value	25 170	75 1000	110 1520	166 1700	148 1650	136 1480	250 1800	121 1300	100 950
sample 6 limit value	25 170	64 1000	134 1520	157 1700	183 1650	155 1480	110 1800	124 1300	131 950
sample 7 limit value	34 190	58 480	132 1200	167 1110	190 980	210 1300	150 1570	110 900	102 840
sample 8 limit value	32 300	77 450	86 630	74 900	103 800	94 1200	124 1100	151 1000	133 930
sample 9 limit value	32 170	303 3130	433 2830	457 3720	683 8670	225 3470	203 3920	165 5480	177 8980
sample 10 limit value	30 370	150 660	317 1210	1000 2100	1356 2580	1389 3090	2389 2740	2100 3660	2433 3530

Table 4. Bioluminescence on the surface of different rubber materials expressed in RLU/cm2

Rubber	Subsequent weeks of the study										
	0	I	II	III	IV	V	VI	VII	VIII	IX	X
sample 1 limit value	37 300	262 1110	1373 980	3633 1330	5283 810	13133 750	12666 930	13000 960	13783 1330	12666 1240	11166 1110
sample 2 limit value	15 150	60 250	51 220	1354 560	2400 810	4297 700	5711 960	4963 900	5342 1200	5140 1200	5336 1330
sample 3 limit value	42 330	360 870	1100 1000	3100 1230	4955 790	6133 1090	5678 2300	4200 2370	3400 1900	3700 2000	3263 2230
sample 4 limit value	37 470	262 980	373 370	2633 530	5283 1310	23166 1160	12666 850	3000 960	13783 3180	12644 2310	13121 2090
sample 5 limit value	14 120	746 760	1666 1220	1433 2590	950 3170	846 2730	963 1430	1117 2110	1063 3630	945 4000	1022 3890
sample 6 limit value	19 140	470 800	3100 1250	7500 1600	10600 1400	12300 850	12150 910	11200 1110	11630 780	10700 980	10500 930
sample 7 limit value	13 70	1750 650	4600 1410	13700 2100	16100 3600	19500 3900	25300 4800	28000 4500	29100 5100	33000 6100	31300 5500
sample 8 limit value	12 90	654 450	1890 630	3750 1140	6570 1500	8590 1560	9100 1680	9600 1700	9500 1680	11900 1520	12300 1460
sample 9 limit value	10 90	850 330	3600 560	7800 800	8500 1000	7900 1200	9100 1450	8900 1600	9500 1700	12400 2200	14500 3100
sample 10 limit value	13 120	900 940	1540 1090	1800 1300	2200 1740	2230 1690	2160 2200	2300 2000	2500 1900	2350 2300	2400 2500

Ten samples made of rubber compounds intended to contact with drinking water were tested. All of them were intended for production of seals and flexible hoses. The testing period was prolonged to ten weeks, what enabled more precise observation of dynamic changes of bioluminescence level determined in swabs coming from the samples surface. Only in two cases (samples no. 5 and 10) the assessment of the material proceeded without reservations. The bioluminescence values from the surface of the remaining 8 samples were significantly higher than analogical values from the surfaces of polymeric materials (PE, PP and PVC). In the course of the results interpretation the application of the final product, was taken into consideration, including the area of the materials surface, which contacts with drinking water in the real application. In case of the products like sealing and rubber pads this surface is usually of the minimal degree and possible microbial growth covers only the connection points inside the installation sealed with these materials. Despite these materials did not meet the requirements for materials contacting with drinking water they were conditionally allowed for that particular kind of application. In cases when the rubber compound was destined for flexible hoses production – the product with significantly larger surface contacting with drinking water than sealing elements – the samples did not obtain positive assessment and they were not allowed to contact with water intended for human consumption.

DISCUSSION

All tested samples of polymeric materials (polypropylene, polyethylene, polyvinyl chloride) demonstrated a low susceptibility to the biofilm formation. The bioluminescence from their surface were within the predetermined acceptable limits, which means they did not exceed ten times the level of bioluminescence determined in swabs taken from the negative control surface. Despite that fact, in some cases, a temporary growth of determined values was observed, which could be related to the migration of the substances like stabilizers and hardeners added to products during their production from the material's surface. These substances may be a potential source of organic carbon for microorganisms, resulting in enhanced growth of their numbers on the materials surface. The studies conducted by *Traczewska* and *Sitarska* [31] also confirmed that the materials of this type due to the emission of the substances stimulating the growth of microorganisms may be susceptible to biofilm formation, and because of that fact, their use in new water supply networks does not provide protection against secondary microbial contamination and corrosion. This particular fact is important in case of the use of poor quality materials characterizing by high emissions of organic substances. The possibility of appearing in the water network microorganisms, that are capable of metabolic degradation of the polymers should additionally be taken into account. However, such a phenomenon occurs at the moment of formation of the biofilm mature form, which is frequently related to poor quality of the material, which contributes to the promotion of microbial colonization of its surface [8]. Also, comparison of the results of the assessment of various organic materials susceptibility, obtained by using a parallel classical microbiological techniques, indicates such character of the most of the polymeric products and materials [1]. However, it should be taken into consideration, that only a small part of this type of materials available on the market was tested. Among them, it can be stated a considerable variety of additives purposed to prolong the life of polymers (plasticizers), giving them a specific physicochemical properties (stabilizers), and other chemical compounds such as dye additions. Each of these substances undergo a migration from the material into the water inside the installation, which can be a potential source of nutrients for microorganisms [24].

The second group of tested materials was rubber, which is generally used for production of sealing elements in fittings and other products in contact with drinking water. Due to complex chemical composition, as well as a very large number of organic additives, which undergo intensive migration to the water, the materials from this group represent usually the largest percentage among all materials and products evaluated negatively. A final assessment of those materials was expanded to include fact, that the sealing elements are usually small in size and their contact with water inside the network is very limited. In spite of this, only two of the tested rubber materials samples have been evaluated without reservations as appropriate to contact with drinking water. In all other cases, the materials were evaluated negatively. In one case, the regular supplier of raw material (a blend of EPDM) has changed its chemical composition without informing about that fact of the manufacturer of the final product. Changes in the chemical composition caused the increased emission of organic substances, which enhanced the growth of microorganisms on the product`s surface, manufactured from raw material of inadequate quality.

Bressler et al. [2] in their study also included the blend of EPDM to materials, which promote the growth of microorganisms, and biofilms formed on the surface of products from these materials which caused secondary microbial contamination of the water network, and additionally might constitute a reservoir of potentially pathogenic bacteria for instance *Pseudomonas aeruginosa.* Similar observations published *Kilb* et al. [11], who described the parts of the installation made of soft rubber materials such as EPDM or NBR as a potential source of secondary microbial contamination of the network inside the building. This is also confirmed by the results of the tests comparing the number of microorganisms in the network supervised by the water producer before it is delivered to the consumer and at the consumer's directly. *Pepper* et al. [28] observed that microbiological contamination of water is generally higher in water samples collected from the consumer, which is the result of the indoor plumbing materials, which promote the growth of microorganisms.

Biofilm formation on materials used for distribution and storage of water intended for human consumption is determined by many factors. Among them the main role play complex relations between water quality and the way of its treatment, and the technical and operating conditions of water distribution systems. The specific technical and chemical properties of the materials and construction products used in the water network are one of the factors that could significantly affect the increase of the phenomenon. The assessment of organic materials used in the storage and distribution of water with regard to their susceptibility to biofilm formation is important for practical reasons. This phenomenon should be taken into consideration in the process of hygienic evaluation of the materials prior to their use in practice. It can be helpful for reducing the scale of the risk associated with biofilm formation and, as a consequence, inadequate microbiological quality of water consumed.

CONCLUSIONS

1. Significant susceptibility to biofilm formation in the group of products made of rubber compounds, including NBR and EPDM compounds, was found.
2. Tested polymeric materials such as polyethylene, polyvinyl chloride, polypropylene, except for several cases, do not revealed susceptibility to biofilm formation.
3. Application of polymer materials for distribution of water intended for human consumption does not fully protect water from secondary microbiological contamination.
4. Further assessment of materials and products contacting with water intended for human consumption is necessary in terms to their susceptibility on biofilm formation.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Assessment of the microbial growth potential of materials in contact with treated water intended for human consumption. Comparison of test methods. KWR 07.068. KIWA Research, 2007.
2. *Bressler D., Balzer M., Dannehl A., Flemming H.-C., Wingender J.*: Persistence of *Pseudomonas aeruginosa* in drinking-water biofilms on elastomeric material. Wat Sci Tech Wat Supp 2009; 9:81-87.
3. BS 6920-2.4; British Standard, 2000. Suitability of non-metallic products for use in contact with water intended for human consumption with regard to their effect on the quality of water. Methods of test. Growth of aquatic microorganisms test.
4. *Chandra J., Zhou G., Channoum M. A.*: Fungal biofilms and actimycotics. Current Drug Targets 2005; 8:887–894.
5. *Czaczyk K., Wojciechowska K.*: Tworzenie biofilmow bakteryjnych – istota zjawiska i mechanizmy oddziaływań. [The formation of bacterial biofilms - the essence of the phenomenon and mechanisms of interactions]. *Biotechnologia* 2003; 3:180–192 (in Polish).
6. *Dohnalik P., Jędrzejewski A.*: Efektywna eksploatacja wodociągów. [Effective exploitation of water supply]. Kraków, Wyd. LEMtech Konsulting Sp. z o. o., 2004 (in Polish).
7. DVGW W270, Microbial Enhancement on Materials to Come into Contact with Drinking Water - Testing and Assessment. 2007.
8. *Flemming, H.C.*: Relevance of biofilms for the biodeterioration of surfaces of polymeric materials. Polym. Degrad. Stabil. 1998; 59**:**309-315.
9. *Grabińska-Łoniewska A., Siński E.*: Mikroorganizmy chorobotwórcze i potencjalnie chorobotwórcze w ekosystemach wodnych i sieciach wodociągowych. [Pathogens and potentially pathogenic microorganisms in aquatic ecosystems and water supply networks] Warszawa, Wydawnictwo Seidel-Przywecki Sp. z o.o. 2010 (in Polish).
10. *Grabińska-Łoniewska A.*: Występowanie grzybów mikroskopowych w systemach dystrybucji wody pitnej i związane z tym zagrożenia zdrowia. [The presence of microscopic fungi in drinking water distribution systems and associated risk]. Gaz, Woda i Technika Sanitarna 2006; 7-8:72-74 (in Polish).
11. *Kilb B., Lange B., Schaule G., Flemming H.-C., Wingender J.*: Contamination of drinking water by coliforms from biofilms grown on rubber-coated valves. Int J Hyg Environ Health 2003; 206:563–573.
12. *Kooij D. van der et al.* CPDW project – Assessment of the microbial growth support potential of products in contact with drinking water. 2003.

13. *Kotowski A.*: Analiza hydrauliczna zjawisk wywołujących zmniejszenie przepływności rurociągów. [Analysis of hydraulic phenomena causing a reduction in flow rate of pipelines]. Ochrona Środowiska. 2010; 1:27–32 (in Polish).
14. *Kwacz M., Zadrosz J.*: Przykłady zastosowań rur z tworzyw sztucznych w sieciach podziemnych. Cz. I. Nowoczesne Budownictwa Inżynieryjne. [An examples of applications of plastic pipes to underground networks. Part. I: Modern Construction Engineering], 2008; 3-4:66-71 (in Polish).
15. *Kwietniewski M., Leśniewski M.*: Materiały przewodów wodociągowych i kanalizacyjnych w aspekcie ich niezawodności. [Material for water pipes and sewage systems in terms of their reliability]. Gospodarka Wodna 2007; 4:158-166. (in Polish)
16. *Kwietniewski M., Rak J.*: Niezawodność infrastruktury wodociągowej i kanalizacyjnej w Polsce. [The reliability of water supply and sewerage infrastructure in Poland]. Warszawa. Wyd. PAN, Komitet Inżynierii Lądowej i Wodnej, Instytut Podstawowych Problemów Techniki. 2010 (in Polish).
17. *Kwietniewski M., Tłoczek M., Wysocki L.*: Zasady doboru rozwiązań materiałowo-konstrukcyjnych do budowy przewodów wodociągowych. [Rules for selection of material and constructional solutions for the construction of water networks]. Bydgoszcz. Izba Gospodarcza Wodociągi Polskie, 2011 (in Polish).
18. *Kwietniewski M.*: Awaryjność infrastruktury wodociągowej i kanalizacyjnej w Polsce w świetle badań eksploatacyjnych. [Failure frequency of water and sewerage infrastructure in Poland according to the exploitation research]. XXV Konferencja Naukowo-Techniczna, Międzyzdroje, 24-27.05.2011 (in Polish).
19. *Långmark J., Storey M. V., Ashbolt N. J., Stenström T-A.*: Accumulation and fate of microorganisms and microspheres in biofilms formed in a pilot-scale water distribution system. Appl Environ Microbiol. 2005; 2: 706–712.
20. *Łebkowska M., Pajor E., Rutkowska-Narożniak A., Kwietniewski M., Wąsowski J., Kowalski D.*: Badania nad rozwojem mikroorganizmów w przewodach wodociągowych z żeliwa sferoidalnego z wykładziną cementową. [Microbial Growth in Cement-lined Ductile Cast-iron Water-pipe Networks]. Ochrona Środowiska. 2011; 3(33):9-13 (in Polish).
21. *Lewandowski Z., Boltz J. P.*: Biofilms in water and wastewater treatment. Treatise on Water Science. 2011; 4:529–570.
22. *Lewandowski Z.*: Structure and function of biofilms. In: *L. V. Evans*, editor. Biofilms: recent advances in their study and control. Amsterdam. Harwood Academic Publishers, 2000, 1-17.
23. *Marshall K.C.*: Growth of bacteria on surface-bound substrates: significance in biofilm development. Recent Advances in Microbial Ecology ed. *Hattori T., Ishida Y., Maruyama Y., Morita R., Uchida A*. Tokyo. Jap. Sci. Soc. Press, 1989, 146-150.
24. *Musz A., Kowalska B.*: Wpływ materiału rurociągów wykonanych z tworzyw sztucznych na jakość wody wodociągowej. Przegląd literatury. Polska Inżynieria Środowiska pięć lat po wstąpieniu do Unii Europejskiej, tom 3. [The influence of the pipelines made of plastic on the quality of tap water. Review of the literature. Polish Environmental Engineering five years after joining the European Union, Volume 3] pod red.: *M Dudzińskiej, L. Pawłowskiego*. 2009; Monografie Komitetu Inżynierii Środowiska; 60:165-178. Lublin (in Polish).
25. *Nawrocki J., Świetlik J.*: Analiza zjawiska korozji w sieciach wodociągowych. [Analysis of corrosion in water supply systems]. Ochrona Środowiska, 2011, 33, 4: 27-40 (in Polish).
26. *Ollos P.J., Huck P. M., Slawson R. M.*: Factors affecting biofilm accumulation in model distribution systems. Journal American Water Works Association. 2003; 1:87–97.
27. Önorm B 5018-1,2002. 2 Prüfung der Verkeimungseignung Trinkwasserrohren, Teil1: Prüfverfahren, Teil 2: Bewertung. Österreichisches Normungsinstitut, 1020 Wien.
28. *Pepper I. L., Rusin P., Quintanar D. R., Haney C., Josephson K. L., Gerba C. P.*: Tracking the concentration of heterotrophic plate count bacteria from the source to the consumer's tap. Int J Food Microbiol. 2004; 92:289–295.
29. *Siemiński M.*: Środowiskowe zagrożenia zdrowia. Inne wyzwania. [Environmental health risk. The other challenges]. Warszawa. PWN, 2007 (in Polish).
30. *Szczotko M., Krogulska B., Krogulski A.*: Badania podatności materiałów kontaktujących się z wodą przeznaczoną do spożycia na powstawanie obrostów mikrobiologicznych. [Investigation of susceptibility of materials contacting with drinking water for microbial growth]. Rocz Panstw Zakl Hig 2009; 60:137-142 (in Polish), PMID: 19803443, https://www.ncbi.nlm.nih.gov/pubmed/19803443
31. *Traczewska T.M., Sitarska M.*: Aspekty mikrobiologiczne stosowania materiałów polimerowych w systemach dystrybucji wody. [Microbiological aspects of polymeric materials application in water distribution systems]. Instal. 2009; 4:38-41 (in Polish).

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