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Original Research

Deterioration of Polymeric Materials Exposed to Metabolic Activity of Microorganisms in a Water Distribution System

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Abstract

Internal surfaces of polymeric (PVC, PE, PP, and PB) pipes were assessed for potential colonization by microorganisms present in a water distribution system. Biofilms that had formed on the surfaces of particular polymer samples were examined by SEM microscopy and Koch's method. Surface damage caused by microbial activity was measured by contact profilometry. PP (1.5×10^6 CFU/cm^2) and PB (4.9×10^5 CFU/cm^2) showed very high potential for developing biofilms on their surfaces. They also displayed considerable surface damage as substantiated by the decrease in the coefficient of profile asymmetry, which amounted to 50% and 85% for PP and PB, respectively.

Keywords: plastic, biofilm, distribution system, microbiological corrosion

Introduction

In recent times, supplying water of desired quality has become an issue of serious concern to many waterworks, since the water produced there frequently deteriorates during transport in the distribution system. It is essential that a water distribution system be considered as a reactor where water undergoes various physicochemical and biochemical changes induced by the variable parameters (water residence time, temperature) of the pipe network [1-3]. Continuing deterioration of the hydraulic conditions in distribution systems is to be attributed to the decline in water demand, the overdimensioning of the water-pipe network, and the poor condition of the pipes, which have been in service over a long period. All of these factors contribute largely to the phenomenon defined as 'recontamination' or 'regrowth' [3-5], which in turn is a major contributor to biofilm accumulation on the internal surfaces of the pipes [2, 3, 6, 7].

Biofilm growth in a water distribution system is highly undesirable, primarily because it enhances corrosion processes as a result of metabolic reactions (microbiological corrosion), especially when the pipes were constructed using conventional materials [8, 9]. Taking into account the wide diversity of aquatic bacteria, there is great potential for adaptive enzymes' activation within the short time of contact between the microorganisms and the pipe material. Another major contributing factor in the extent of biocorrosion is the presence of fungi, whose metabolic activity enhances pipe degradation [10-12].

Biocorrosion phenomena also are influenced by the deposits that develop in a water distribution system, which frequently becomes an ideal medium supporting biofilm growth. Biofilms accumulating on the internal surfaces of water pipes account for 1 to 12% of the organic matter in

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such a deposit (1% of this organic matter being equivalent to 1.2×10^8 CFU) [13].

In recent years plastics have replaced many conventional materials, also those used for the construction of drinking water distribution systems. Polyvinyl chloride, polyethylene, polypropylene, and polybutylene are widely used not only in plumbing fittings, but also in water mains. Their widespread use is attributable to the advantages they offer when compared with traditional materials: light weight, chemical stability, and simple assembling techniques [14].

The potential for colonization by microorganisms largely depends on the structure of the material's surface. That is why water pipes made from plastic materials were assumed to be immune to biofilm growth mainly because of their poor surface roughness. This assumption, however, was found to be unjustified [12, 15-17].

Taking into account the technological and microbiological threats to which water-pipe networks are exposed, it seemed advisable to examine the response of the materials used in water distribution systems to the threats mentioned. The continuing use of new materials in the construction of a sanitary system, as well as the continuing advances in the technology of producing them, raises many problems that have to be solved. Among them the rate of biofilm development and the effect of the biofilm on the material being colonized deserve particular consideration. In this work, attempts were made to assess the extent of biofilm growth on polymeric materials and the microbiological corrosion caused therein.

Materials and Methods

Materials

The material samples chosen for this study were sections of fresh pipes made from the following synthetic polymers used in drinking water distribution systems: unplastified polyvinyl chloride PVC-U (polyvinyl chloride/PVC), polyethylene HDPE-100 (polyethylene/PE), polypropylene PP-R type 3 copolymer (polypropylene/PP) and poly-1-butylene (polybutylene/PB).

Experiments were conducted in a fluid-flow system, using samples with an exposure surface of 30 cm^2 each. The samples used in the experiments performed under shake-flask conditions were 25×20 mm in size and had a thickness of 4 mm, which is equivalent to an exposure surface of 13.6 cm^2.

Biofilm Culture in a Fluid-Flow System

Experiments were conducted in two series. In the first series, polyvinyl chloride and polyethylene were tested for the potential of promoting microbial growth on their internal surfaces; in the second series, these tests were performed with polypropylene and polybutylene. The experimental series had durations of 150 days each, and were performed in duplicate.

The laboratory set-up consisted of two 40×80×50 mm reactors made of stainless steel. Two 30 cm^2 plates of the same polymeric material were placed in each reactor and arranged parallel to water flow. The set-up was fed with tap water. Water flow velocity in the laboratory set-up was 0.2 m/s. The water distribution system was fed with surface water and infiltrative water.

Numbers of mesophilic and psychrophilic bacteria were monitored in inlet and outlet water throughout the experiment.

Upon termination of exposure, the microorganisms that had colonized the surface of the material samples were released using the cavitation method, whereas their number was determined by Koch's method. The polymer samples were examined by scanning electron microscopy while simultaneously taking photographs of the biofilm.

Biofilm Culture under Shake-Flask Conditions

Sterile samples of polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), and polybutylene (PB), 25×20 mm in size and 4 mm in thickness, were placed in a 200 cm^3 volume of minimal medium [18], inoculated with a 10 cm^3 volume of a mixture of microorganisms separated from tap water. Inoculum for the shake-flask culture prepared by filtration of a 3 dm^3 volume of tap water through a sterile cellulose filter (0.2 μm pore size). The microorganisms retained on the filter were released into a 50 cm^3 volume of physiological solution by cavitation (5 min. in an ultrasonic bath). A 10 cm^3 volume of microbial suspension was inoculated to 200 cm^3 of minimal medium and incubated at 22±2ºC for 72 h. After incubation the number of microorganisms in the inoculum was 1×10^4 CFU/cm^3.

The total number of microorganisms in the culture amounted to 5×10^2 CFU/cm^3. The polymer samples were exposed to microbial activity for 40 days at 22±2ºC under aerobic conditions with slow mix; after that the extent of surface colonization was assessed.

After 40 days the biofilm that had formed on the surfaces of the polymeric materials was removed by cavitation and the number of microorganisms in the solution was determined using Koch's method. Biofilm density was established (after suitable conversions) based on the number of microorganisms colonizing a unit surface.

Before and after exposure, the texture of the polymer's surface was measured by contact profilometry in order to determine structural changes (pits/defects).

Assessment of Surface Structure and Biofilm Density by SEM Microscopy

Surface structure of the polymeric materials (before and after exposure to metabolic bacterial activity) and biofilm densities were assessed from SEM micrographs (Leo Zeiss 435 Vp microscope, Oberkowe). The samples were stabilized with 2.5% glutaraldehyde in phosphate buffer, dewatered at 5ºC (in ethanol rinsing series from 25 to 100%), dried, and coated with gold (Scancoat 6 made by Oxford).

Upon termination of the coating procedure the samples were examined under a SEM microscope and their photographs were taken.

Quantification of Microorganisms

After 150 days of culture in the fluid-flow system and 40 days of culture under shake-flask conditions, the biofilms that had accumulated on the sample surfaces were removed by cavitation. The samples were first placed in flasks (each containing a 50 cm^3 volume of physiological solution), and then in an ultrasonic bath for 20 min. The suspensions obtained via this route were diluted and plate counts were carried out (in triplicate) using nutrient agar enriched with sodium chloride and meat extract [19]. Enrichment of the standard nutrient agar allowed the growth of fastidious microorganisms. Samples were incubated at 22±2ºC by 72h and 36±2ºC by 48h.

Measurement of Surface Texture

Surface roughness was examined before and after exposure of the polymeric materials to a mixture of microorganisms isolated from tap water.

Measurements were performed by the contact profilometry method, using a 3D optical surface profiler (Form Talysurf-120 L, Taylor Hobson) with a conic needle (needle parameters: angle, 900; radius of vertex rounding, 2 μm; needle pressure on material tested, 0.7 mN). Fitted with special software, the device enabled mathematical analysis of the results obtained from numerous measurements and presented them as single numerical values.

Results and Discussion

Biofilm Culture in a Fluid-Flow System

The aim of this study was to examine the potential for biofilm development on the internal surfaces of PVC, PE, PP, and PB pipes, as well as the impact of microbial metabolic activity on these polymeric materials. Investigations reported in the literature suggest that biofilms may form within three weeks [20]. Zacheus et al. obtained a 7.7×10^5 CFU/cm^2 biofilm on the surface of PVC after three weeks, whereas Codony et al., who neutralized chemical disinfectants with sodium thiosulphate, obtained a 1×10^7 CFU/cm^2 biofilm within 15 days [21, 22].

In our study, the number of psychrophilic bacteria in the water fed to the experimental set-up varied from 0 to 1×10 CFU/cm^3, and that of mesophilic bacteria ranged between 0 and 4.1×10 CFU/cm^3, with incidental increase to 6.1-8.1×10 CFU/cm^3. The number of psychrophilic bacteria in outflow varied from 1 to 1.1×10^3 CFU/cm^3, with incidental increase to 1.6×10^4 CFU/cm^3, and mesophilic bacteria in the 0-3×10^3 CFU/cm^3. Quantitative analysis of bacteria removed from the surfaces of the polymeric materials revealed that polyvinyl chloride had the lowest potential for being colonized by microorganisms; the number of bacteria per 1 cm^2 of polymer surface was the lowest among the materials tested (Table 1). Although the number of psychrophilic bacteria that had developed on PVC was 3.5×10^3 CFU/cm^2, it was comparable with that on polyethylene (9.3×10^3 CFU/cm^2), but the number of mesophilic bacteria on PVC differed significantly from the numbers observed on PE, PP, and PB. Thus, for polyvinyl chloride the number of mesophilic bacteria was only 8×10 CFU/cm^2, whereas for PE, PP, and PB it ranged between 8×10^2 and 2.2×10^3 CFU/cm^2. The highest number of psychrophilic bacteria per 1 cm^2 of surface was noted on polypropylene (1.5×10^6 CFU/cm^2), but the number of mesophilic bacteria obtained for this polymer was similar to that observed on PB. The highest number of mesophilic bacteria, which amounted to 2.2×10^3 CFU/cm^2, was noted on polyethylene. The comparison of PP and PB surfaces revealed no significant differences in the counts of mesophilic bacteria, which varied between 8×10^2 and 1.2×10^3 CFU/cm^2.

Table 1. Number of bacteria per 1 cm^2 of surface, accumulated on the polymeric materials after 150 days.

Material	PVC	PE	PP	PB
Number of psychrophilic bacteria [CFU/cm^2]	3.5×10^3	9.3×10^3	1.5×10^6	4.9×10^5
Number of mesophilic bacteria [CFU/cm^2]	8×10	2.2×10^3	1.2×10^3	8×10^2

All of the pipe materials tested were found to be colonized by biofilm, but the extent of colonization differed from one polymer type to another. These differences are attributable not only to the number of psychrophilic and mesophilic bacteria, but also to the proportion between them. In the case of PP and PB, the number of psychrophilic bacteria accounted for 99.92% and 99.84% (respectively) of the total bacterial population that had colonized the polymer surface. For polyvinyl chloride the proportion was 97.77%, and for polyethylene only 80.87%. A reverse trend was observed with mesophilic bacteria, whose number accounted for less than 0.2% of overall bacterial population in the case of PP and PB, and for approximately 20% in the case of polyethylene. Since the water to which the samples were exposed was of comparable quality throughout the experiments, biofilm density was influenced by the type of the polymeric material rather than the composition of the water.

SEM microscopy not only confirmed the finding that the extent of colonization by microorganisms was the lowest on polyvinyl chloride, but also detected small amounts of fungi in the bacterial population. The SEM micrographs obtained allowed general comparison of the biofilm's spatial structure. In the case of PVC, this structure was sufficiently smooth and displayed no local agglomerates of microorganisms. Microbial numbers were lower, which should be attributed to the lower biofilm density. SEM micrographs also revealed the presence of inorganic deposits with the potential for augmenting surface rough-

ness, which may trigger an increase in cell adhesion to the polymer with time of pipe service.

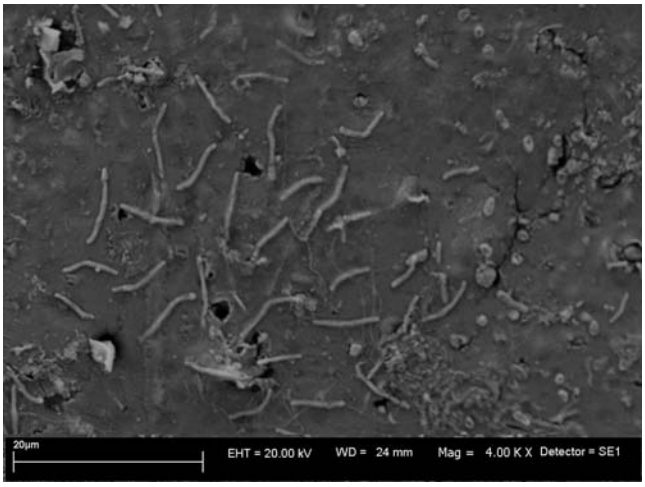
Compared with polyvinyl chloride, larger counts of microorganisms, as well as a wider diversity of microbial morphology, were noted on the surfaces of PE, PP, and PB. In the case of polyethylene, microorganisms were almost evenly distributed, which seems to be due to the character of the polymer's surface. The largest and the most spatially differentiated biofilm was observed on PP and PB surfaces, which exhibited distinct agglomerates varying in cell morphology and density. SEM micrographs showed that fungi were present in these biofilms.

Fig. 1 gives examples of biofilm that had developed on the surfaces of the polymeric materials tested.

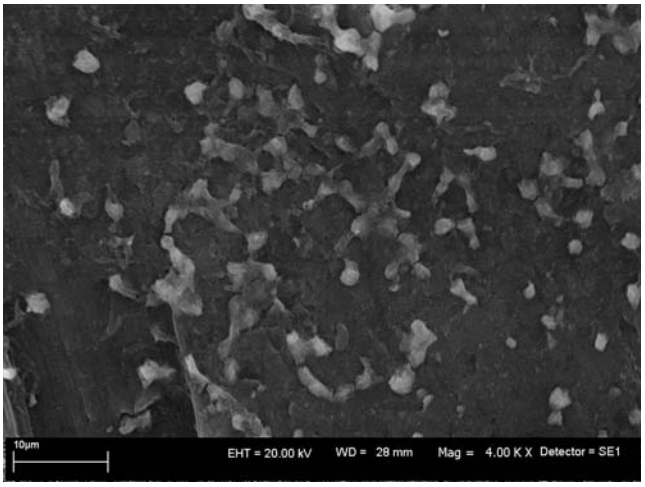
Planktonic cells synthesise only small amounts of extracellular polymeric substances (EPS). While biofilm is forming, EPS production increases, but the number and chemical diversity of these substances depend on the environment and the type of polymer. EPS may, for example, perform integrating functions and thus stabilize the structure of the biofilm. Owing to the high content of enzymes and other substances facilitating decomposition of complex chemical compounds present in the polymer into simple ones, EPS may provide a nutrient source for further biofilm growth. This is a serious threat to a drinking water distribution system and, consequently, to public health [12, 23].

The literature contains references to various mechanisms underlying the occurrence of material corrosion. Those triggered by the presence of microorganisms are part of pitting corrosion. The inherent characteristic of pitting corrosion is the formation of pits on the entire polymer surface. The pits differ in number and size, and are influenced by the number and diversity of the bacteria that colonize the polymer [22]. This is to be attributed to the uneven distribution of the microorganisms on the polymer surface, which stimulates growth of local agglomerates. In many instances local domination can be observed of certain bacterial genera or species that have the ability to synthesize substances differing largely in toxicity to polymeric materials.

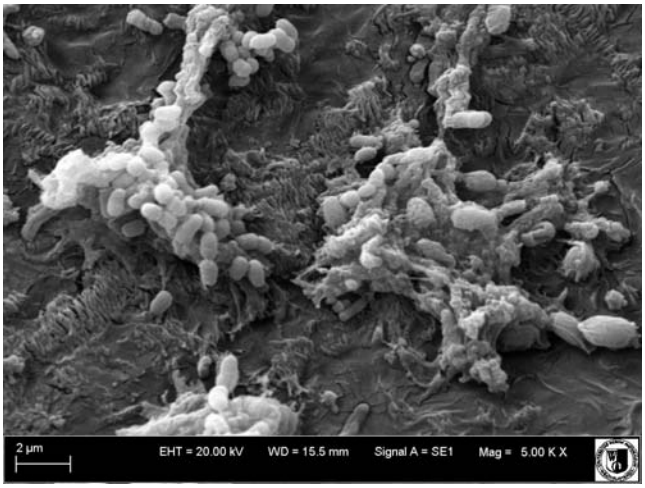
This work reports changes observed on the internal surfaces of polymer pipes as a result of metabolic activity of the microorganisms that colonize them. Relevant data are visualized in Figs. 2 to 5.



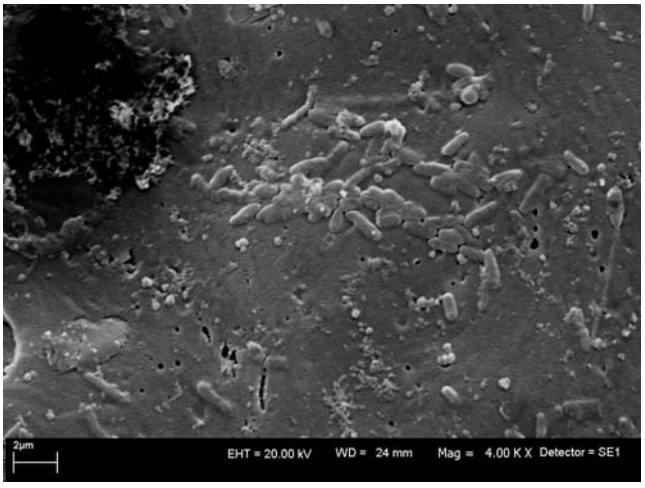
(a) PVC (magnification, 4000x)



(b) PE (magnification, 4000x)



(c) PP (magnification, 5000x)



(d) PB (magnification, 4000x)

Fig. 1. Biofilms developed on polymers during experiments in the fluid-flow system after 150 days.

Pits observed on the PVC surface are small but numerous. Initially, some part of the surface structure bore a resemblance to 'stripes of fibres'. As a result of damage, some of those stripes broadened, others disappeared (Fig. 2).

As for the examination of the PE surface, it is essential to take account of two issues: the effect of bacterial and fungal metabolites, and exposure to chlorinated water disinfectants. Chlorine exposure is to be blamed for deteriorating the technological parameters of polyethylene. Chromatographic and differential scanning calorimetric examinations (DSC) performed by Hassinen et al. revealed considerable degradation, with severe damage to the surface and crystalline structure of the polymer. It is therefore advisable to consider potential occurrences of turbulence corrosion, specifically at the initial stage of the pipeline's service life. Such corrosion occurs primarily during nocturnal water stagnation, with significant decline in water demand and noticeable release of chemical reaction products. Over this period the pipe material is exposed to harmful substances [24]. Our present study disclosed dramatic changes in the surface structure of polyethylene. After 150 days of exposure, the initially smooth surface of the polymer was rough, and displayed numerous defects and mineral deposits that acted as an ideal medium for supporting colonization by microorganisms (Fig. 3).

The influence of biofilm density and maturity on the extent of surface damage was particularly distinct with PP and PB. The most significant changes were noted when their surfaces had been colonized by very dense biofilm with wide microbial diversity. For PP the number of psychrophilic bacteria per 1 cm^2 of surface amounted to 1.5×10^6 CFU, while that for PB was 4.9×10^5 CFU. Biofilm activity was also a contributory factor in the appearance of numerous pits and amorphous deposits. This finding has been confirmed by Liu et al., who observed (via SEM microscopy) a roughness-induced increase in the number of microorganisms on pipe surfaces [25]. In our study, upon termination of the cavitation process, the surfaces of PP and PB displayed single microbial agglomerates, whose adhesion to both the materials (via EPS released by the bacteria) was too strong to enable their removal by treatment in ultrasound (Figs. 4 and 5).

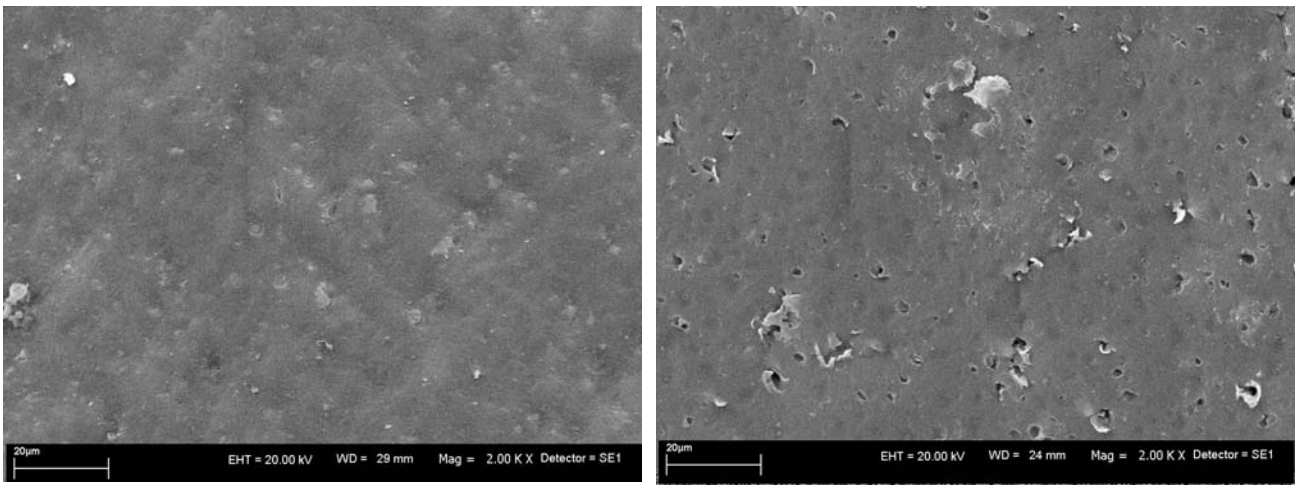


Fig. 2. SEM micrographs of fresh PVC samples before biofilm removal (on the left; magnification, 2000x) and after biofilm removal (on the right; magnification, 2000x).

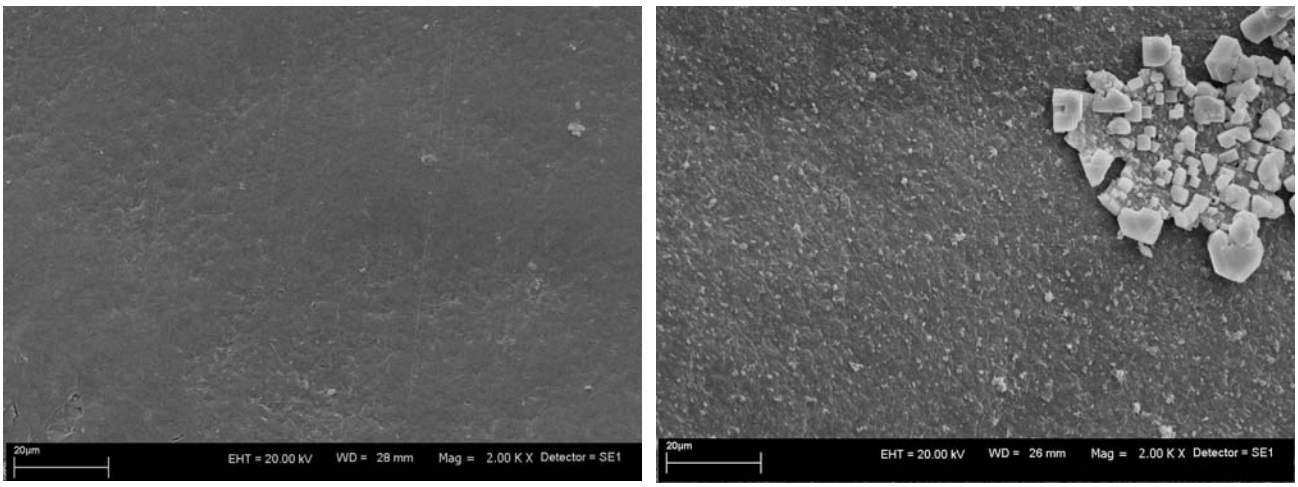


Fig. 3. SEM micrographs of fresh PE samples before biofilm removal (on the left; magnification, 2000x) and after biofilm removal (on the right; magnification, 2000x).

Biofilm Culture in Shake-Flask Experiments

Shake-flask experiments were carried out for a better understanding of how the metabolism of the microorganisms in the drinking water distribution system affect the surfaces of polymeric pipes. The polymeric materials were analyzed for surface roughness before and after incubation with a suspension of microorganisms. Then the changes observed, as well as the relation between these changes and the number of bacteria that had colonized the material samples, were quantified.

The polymeric materials examined were placed in a minimal medium containing a 5×10^2 CFU/cm^3 volume of bacteria for a time span of 40 days. Then the biofilm was removed by the cavitation method (bacterial cells being released into the physiological solution). Using standardized quantitative microbiological analyses from the solution, upon appropriate conversions, the biofilm that had colonized particular polymeric materials was quantified. The results are summarized in Table 2.

Also in these experiments, polyvinyl chloride showed the lowest potential for being colonized by microorganisms. The number of psychrophilic bacteria detected on its surface was 2.8×10^4 CFU/cm^2, which is much less compared with the values obtained for the other polymers: 1.6×10^5 CFU/cm^2 for PE, 1.4×10^5 CFU/cm^2 for PP, and 1.5×10^5 CFU/cm^2 for PB. The number of mesophilic bacteria amounted to 1.2×10^4 CFU/cm^2 and was comparable with the values obtained for PE, PP, and PB. On the surfaces of polyethylene, polypropylene, and polybutylene the numbers of bacteria incubated at 22±2ºC and 37±2ºC were similar. The biofilm that had formed on the surfaces of the polymeric

Table 2. Number of bacteria per 1 cm^2 of surface, accumulated on polymeric materials after 40 days of shake-flask experiments.

Material	PVC	PE	PP	PB
Number of psychrophilic bacteria [CFU/cm^2]	2.8×10^4	1.6×10^5	1.4×10^5	1.5×10^5
Number of mesophilic bacteria [CFU/cm^2]	1.2×10^4	6×10^3	4×10^3	1×10^4

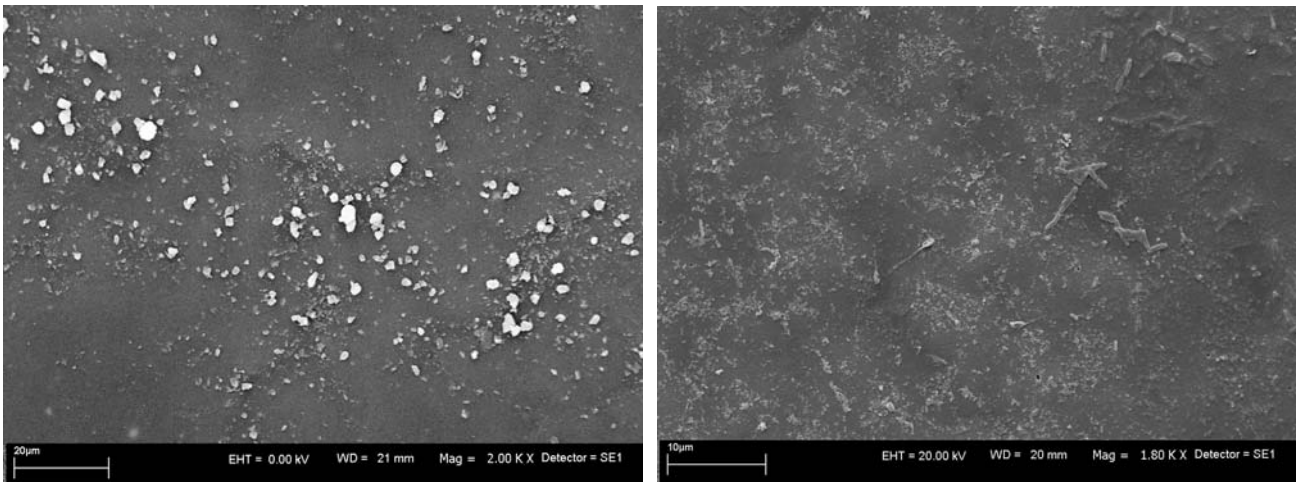


Fig. 4. SEM micrographs of fresh PP samples before biofilm removal (on the left; magnification, 2000x) and after biofilm removal (on the right; magnification, 1800x).

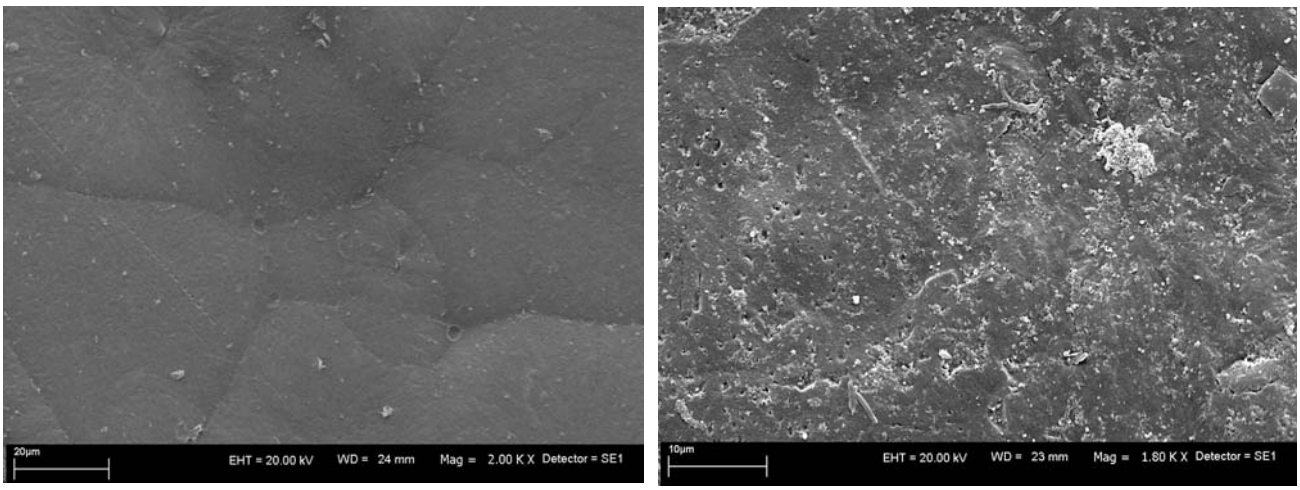


Fig. 5. SEM micrographs of fresh PB samples before biofilm removal (on the left; magnification, 2000x) and after biofilm removal (on the right; magnification, 1800x).

materials was characterized by a high proportion of psychrophilic bacteria, which ranged between 93% and 97% in all but one of the polymers. The only exception was in polyvinyl chloride, where the proportion approached 70%.

Upon biofilm removal the samples were analyzed for texture changes on their surfaces with reference to fresh samples. The cutting plane was set to a height of 0.37 μm under the reference plane, which accounted for 40 to 45% (4 to 6 μm when counted from the deepest valley) of the total height. Taking into account the size of the bacterial cells (approximately 0.5 μm), it was expected that the greatest changes induced by the metabolic activity of the bacteria would occur at that level of the cutting plane. Roughness was measured using surface sections of about 4 mm^2, the same for each of the polymers examined. The results are shown in Table 3.

Polyvinyl chloride surface showed the highest skewness (S_{sk} = 0.76). That this is an indication of a greater profile asymmetry with respect to the reference plane can be inferred from the kurtosis (S_{ku}) value of 2.89 (< 3.0), which describes the broadening of profile ordinate distribution with respect to the mean plane (frequency of vertex occurrence). For polyethylene the skewness value was lower (S_{sk} = 0.14), but kurtosis remained constant with values lower than 3.0. The greatest asymmetry of ordinates was that of polypropylene, but the profile oblateness coefficient took the value of 3.29 (> 3.0). Polybutylene was characterized by a skewness of 0.33 and a kurtosis of 3.17. The great asymmetry of ordinates and the high coefficient of oblateness make it clear that among the polymers tested, polybutylene displayed the highest surface roughness.

Exposure to bacterial flora brought about a reduction in skewness, which was an indication of a decrease in peak height and, consequently, of a reduction in surface roughness due to material defect. The change in skewness was particularly distinct in polybutylene (85.2 %) and was concomitant with a decrease in kurtosis by 7.3%. Polybutylene had also been colonized by large numbers of psychrophilic and mesophilic bacteria (1.5×10^5 CFU/cm^2 and 1×10^4 CFU/cm^2, respectively). The coefficient of profile asymmetry for PVC and PE decreased by approximately 70%. Kurtosis decreased by 13.1% for polyvinyl chloride and increased by 1.1% for polyethylene. For polypropylene, S_{sk} decreased by 50.0%, whereas S_{ku} increased by 3.3%. An increase in the height of the greatest peak (S_p) and in the depth of the greatest valley (S_v) was observed on the surfaces of polyvinyl chloride and polybutylene. Thus, for PVC, S_p increased by 0.26 μm (4%) and S_v by 0.17 μm (3.1%); for polybutylene, the increase in S_p totalled 0.43 μm (12.9 %) and that in S_v was 0.09 μm (lower than 2%). A reduction in the highest peak and an increase in the depth of the deepest valley were observed with polyethylene (where S_p decreased by 1.14μm (20.1 %) and S_v by 0.64μm (12.6%)) and polypropylene (where S_p decreased by 0.68μm (11.7%) and S_v by 0.96 μm (15.7 %)).

Differences in the arithmetic mean of the deviations from the profile (S_a) before and after exposure to the microflora of the biofilm ranged from 0.01 to 0.17 μm. For PVC and PB the value of this parameter increased, which is attributable to the relatively small changes (defects) that appeared on the surfaces of the two materials as a result of biodegradation. A significant difference was noted in the polyethylene sample, where the value of S_a decreased from 1.34 μm to 1.17μm (i.e. by more than 11%), while the other polymer samples showed an increase in the value of this parameter, which ranged between 0.9% and 3.8%. No significant changes were observed on the surface of polypropylene.

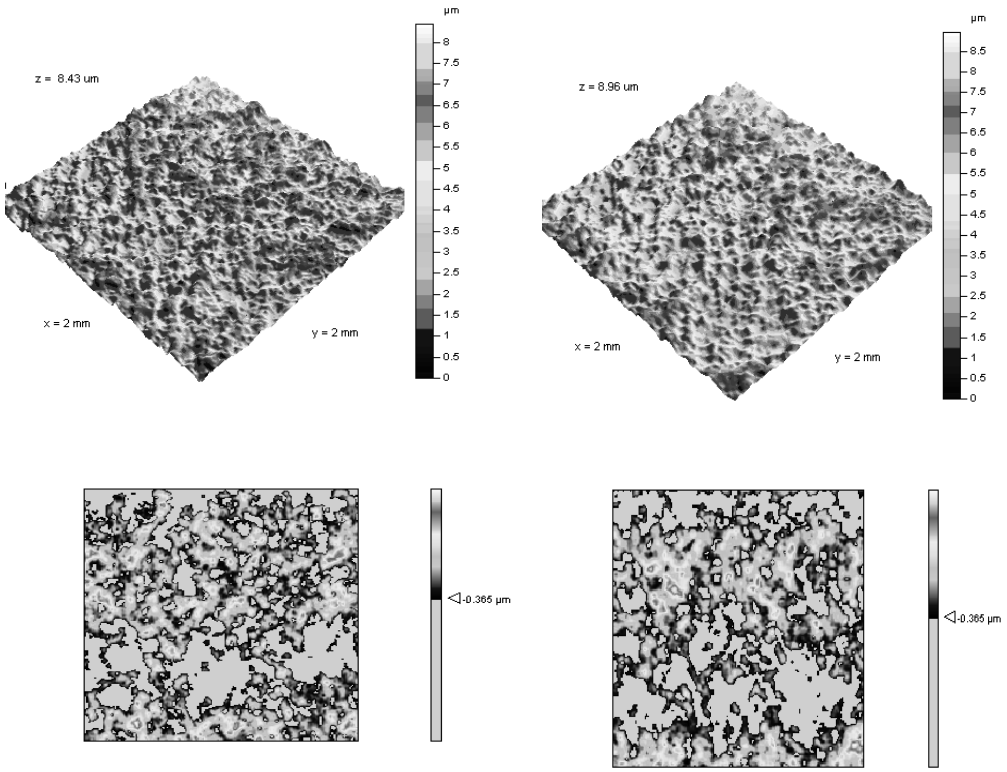
Table 3. Roughness parameters for polymer pipes before and after exposure to microorganisms.

	Roughness parameter	Fresh pipe	Upon exposure to microflora of tap water	Extent of change [%]
PVC	S_a	1.56	1.62	3.8
	S_q	1.91	1.96	2.6
	S_{sk}	0.76	0.24	-68.4
	S_{ku}	2.89	2.51	-13.1
	S_p	6.48	6.74	4.0
	S_v	4.86	5.01	3.1
	S_z	11.3	11.8	0.4
PE	S_a	1.34	1.17	-11.2
	S_q	1.65	1.44	-12.7
	S_{sk}	0.14	0.05	-68.6
	S_{ku}	2.66	2.69	1.1
	S_p	5.68	4.54	-20.1
	S_v	5.06	4.42	-12.6
	S_z	10.7	8.96	-16.3
PP	S_a	1.15	1.16	0.9
	S_q	1.47	1.49	1.4
	S_{sk}	0.10	0.05	-50.0
	S_{ku}	3.29	3.40	3.3
	S_p	5.80	5.12	-11.7
	S_v	6.12	5.16	-15.7
	S_z	11.9	10.3	-13.4
PB	S_a	0.80	0.82	3.4
	S_q	1.01	1.02	1.0
	S_{sk}	0.33	0.05	-85.2
	S_{ku}	3.17	2.94	-7.3
	S_p	3.33	3.76	12.9
	S_v	5.10	5.19	1.8
	S_z	8.43	8.94	6.0

The extent and structure of change on the internal surface of the polymer pipe also can be assessed by measuring the number and volume of islands before and after exposure. As for polyvinyl chloride, the number of islands prior to exposure was 72, their mean height, mean surface area, and the ratio of mean height to mean surface area amounted to 0.353 μm, 0.02 mm^2, and 19.4, respectively. Upon incubation and biofilm removal, the number of islands decreased to 18, while mean height and mean surface area increased to 0.809 μm and 0.083 mm^2, respectively. Reduction in the number of islands, which was paralleled by an increase in their height and surface area, resulted from the change in the structure of the polymer surface. The biofilm that had formed increased the depth of the valleys. The decrease in the number of islands might have been caused by their merging into one another as a result of metabolic activity of the microorganisms that were present in the biofilm. Polyvinyl chloride was the least colonized polymeric material. It is essential to note, however, that the microorganisms on its surface followed an almost even distribution pattern. An exposure-induced decline in the number of islands also was noted on the polyethylene surface, where it decreased from 65 to 24. Mean island height increased nearly twofold: from 0.567 μm to 0.909 μm (a similar effect being observed on the PVC surface), whereas the increase in mean island surface was slightly less pronounced: from 0.0141 mm^2 before exposure to 0.0402 mm^2 after exposure. As a result of microbial activity, the surface of the polymer was flattened, single islands merged to form larger ones, and all additional valleys between them increased in depth. Such changes point towards the presence of biofilm as a contributing factor in material loss. Polypropylene appeared to be equally sensitive to the influence of microorganisms. The number of islands on its surface decreased. Their height and surface area, however, did not change as significantly as on PVC and PE, increasing only by 0.1 μm and 0.0253 mm^2, respectively.

For polybutylene the three-dimensional image disclosed 57 islands, whose initial number was 36. The increase in island height by only 0.081 μm was insignificant. In contrast to the other polymers, a decrease was observed in the surface area value, which fell from 0.0734 mm^2 to 0.0438 mm^2. The ratio of mean height to mean surface area increased from 5.96 to 11.8. These changes augmented the number of islands and sharpened their edges, thus contributing to a noticeable rise in surface roughness. Taking account of the fact that polybutylene was densely colonized by microorganisms, it can be assumed that the changes mentioned here were induced by their metabolic activity.

The extent of change in the surface of polymeric materials was illustrated using polybutylene as an example (Fig. 6).



Island number	36	Island number	57
Mean island volume	67709 μm^3	Mean island volume	43155 μm^3
Mean island height	0.437 μm	Mean island height	0.518 μm
Mean island surface area	0.0734 mm^2	Mean island surface area	0.0438 mm^2
Mean height/mean surface area ratio	5.96 μm/ mm^2	Mean height/mean surface area ratio	11.8 μm/ mm^2

Fig. 6. Changes in the texture of polybutylene surface induced by the presence of biofilm.
(a) Three-dimensional image: fresh PB pipe, before biofilm removal (on the left) and after biofilm removal (on the right)
(b) Changes in island number and volume: fresh PB pipe, before biofilm removal (on the left) and after biofilm removal (on the right)

Upon exposure to the microflora in the drinking water distribution system, all of the polymeric materials exhibited changes on their surfaces. Polyvinyl chloride and polybutylene developed an increase in surface roughness, thus enhancing their potential for being easily colonized by microorganisms, including those with poor adhesive properties.

The biofilm growth and rise in surface roughness observed in this study provide evidence to suggest that polymers are not immune to biodegradation.

Conclusions

1. Polymeric materials used in drinking water distribution systems promote biofilm development. This has been substantiated by analysis of the numbers of microorganisms that colonized the polymeric materials tested, and also by SEM examinations of biofilm formed.
2. The potential of the polymer to promote biofilm growth can by ranked in the following order: polybutylene > polypropylene > polyethylene > polyvinyl chloride.
3. The metabolic activity of the microorganisms contained in the biofilm suggests the occurrence of microbiological corrosion/biodegradation. This can be implied from the measured values of surface roughness, as well as from the SEM micrographs of the polymer surfaces where pits/defects occur.
4. The structure of the polymer surface influences the extent of colonization.

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