Environment Protection Engineering

Vol. 35 2009 No. 1

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DEVELOPMENT OF BIOFILM ON SYNTHETIC POLYMERS USED IN WATER DISTRIBUTION

The susceptibility of synthetic polymers to an overgrowth with microbial biofilm used in drinking water distribution system is assessed by calculating the number of microorganisms developing biofilms on specific materials and the analysis of the SEM images of biofilms.

1. INTRODUCTION

A technological progress over the last few years has resulted in an increasing application of synthetic polymers in various aspects of human life. These materials are at present successfully used also in water distribution systems where their percentage is steadily rising [1], [2].

The initial studies indicated that such synthetic polymers as PE or PVC demonstrate poor surface roughness which is a direct cause of their impermeability to microorganisms such as bacteria and fungi. Their low susceptibility to the development of microbial film is comparable to that of copper which demonstrates antiseptic qualities [2], [3].

The latest research proves that these polymers are not resistant to microorganisms and their smooth surfaces only slow the development of the first layer of biofilm; the next stages of its development can be faster than in the case of other materials, because the substances used for their production such as fixers, stabilizers or hardeners can be a source of carbon for bacteria and thus stimulate their growth in biofilm. The biodiversity of biofilm being developed on specific materials also varies because of their different roughness which frequently leads to the development of the first layer of biofilm from the bacteria with the best adhesive properties [1], [3].

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The biofilms which develop in the water distribution systems pose at present the problem for many water providers. The oversized piping, old corroded sections and constantly decreasing water intake causing its stagnation are the factors which contribute to the development of microorganisms on piping system walls and fitting elements. This phenomenon results in a worse quality of water delivered to consumers, which is defined as the secondary contamination of water. The bacteriological contamination and the changes of both physical and chemical parameters of water pose danger to human health [4]–[8].

2. RESEARCH METHODS

The objective of research was to evaluate the rate of the development of microorganisms which are present in tap water, on synthetic materials used for water supply systems.

The rate of the biofilm development on PCV, PE, PB and PP surfaces was tested at the station located in the laboratory of the Institute of Environmental Protection Engineering of Wrocław Technical University. The samples of the materials tested (30 mm × 35 mm and 4 mm thick) were placed successively in a reactor made of acid-resistant stainless steel for the period of about 160 days. The reactors were then fed with tap water from the cold water intake point located in Wrocław water distribution system, and the water flow rate was 0.2 m/s.

Before being placed in the reactor, the material samples were analysed in the scanning electron microscope in order to compare the structure of surface of the polymers used in the tests and after the end of their 160-day contact with tap water. After that time the biofilm which had developed was removed from polymers by the ultrasonic method and the material was again subjected to microscopic analysis in order to show the changes which took place on the surface of the materials as a result of metabolic activity of the microorganisms developing the biofilm. The number of the microorganisms which appeared on the surface of the material were calculated per area of the material by means of standard microbiological methods. The substrate enriched with agar was used to calculate the total number of bacteria, whereas the Sabouraud substrate was used to calculate the total number of fungi and mold.

3. DISCUSSION

SEM images demonstrate the differences in the polymer surface structures. Figures 1, 2, 3 and 4 show SEM images of the surface of fragments of new pipelines made respectively of polyvinyl chloride (PCV), polyethylene (PE), polybutene (PB) and polypropylene (PP).

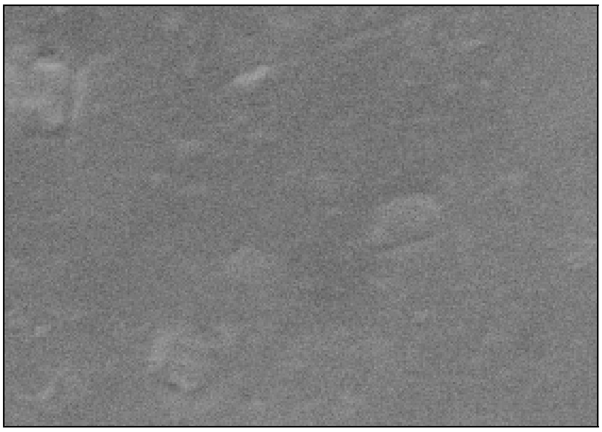

Fig. 1. SEM image of surface of new pipeline made of PVC (magnification of 6 000×)

The most regular structure is demonstrated by polyvinyl chloride whose surface resembles a “truss” with individual strands of the material making oblique straps similar to natural fibers. Slight irregularities in depth and shape of this structure are visible.

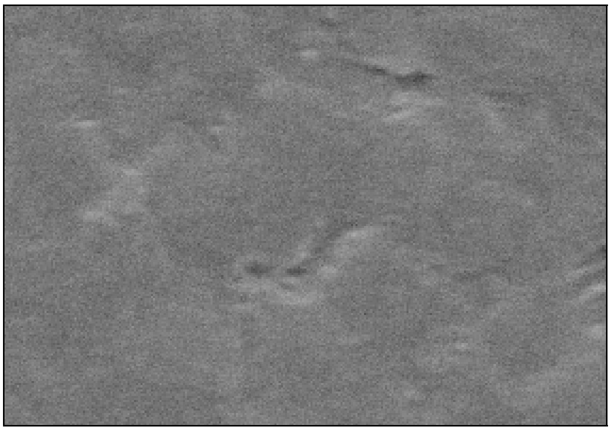

Fig. 2. SEM image of surface of new pipeline made of PE (magnification of 6 000×)

The surface of polyethylene is different, as only under higher magnification its folding resembling swelling is visible.

On the surface of polybutylene we observed polygonal structures connected to make an integrated whole with significant irregularities between them; additionally, numerous crystals made the surface rougher.

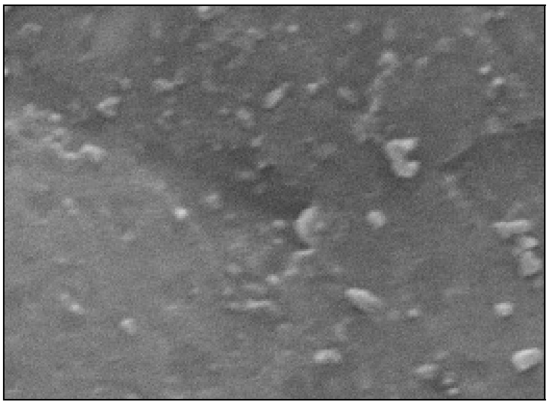

Fig. 3. SEM image of surface of new pipeline made of PB (magnification of 6 000×)

Polypropylene, which resembles a "file" as its rather smooth surface is covered with numerous particles/crystals of different sizes and shapes scattered highly irregularly, seems to be the material of the highest roughness. These crystals are much greater and more numerous than those in the case of polybutylene.

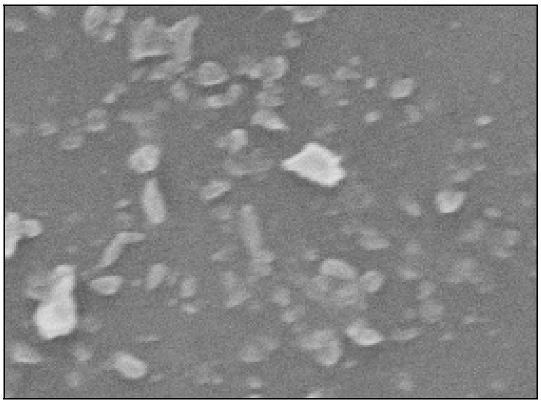

Fig. 4. SEM image of surface of new pipeline made of PP (magnification of 6 000×)

Figures 5–8 show SEM images of synthetic material samples covered with biofilm after a 160-day period of their contact with tap water.

A close observation of the biofilms which developed on the materials reveals the distinctive differences in their structure, depending on the substrate on which they developed. A whole surface of polyvinyl chloride seems to be uniformly covered with the biofilm, as the surface of polyethylene and polybutylene, where additionally few conglomerates of bacteria are also visible, whereas the biofilm on the surface of polypropylene is most diversified spatially.

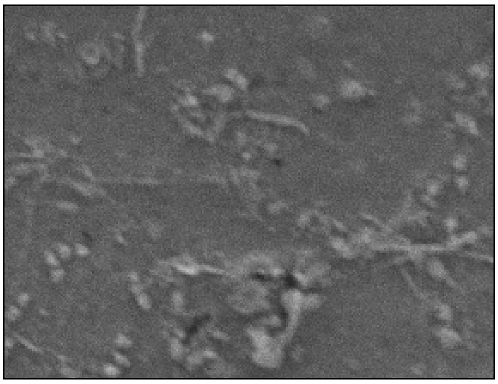

Fig. 5. SEM image of biofilm developed on surface of new pipeline made of PVC (magnification of 2 000×)

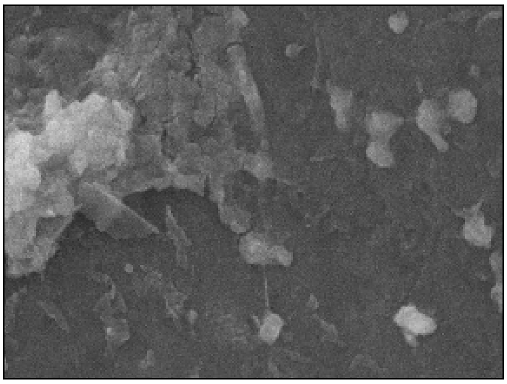

Fig. 6. SEM image of biofilm developed on surface of new pipeline made of PE (magnification of 2 000×)

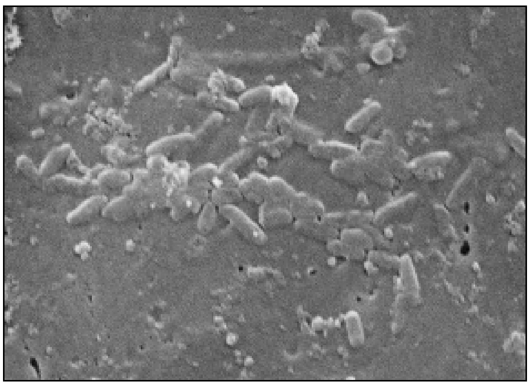

Fig. 7. SEM image of biofilm developed on surface of new pipeline made of PB (magnification of 1 800×)

On the basis of figures 5–8 it can be seen that the biofilm developed on polybutylene and polypropylene has a highly structured surface with numerous fungi.

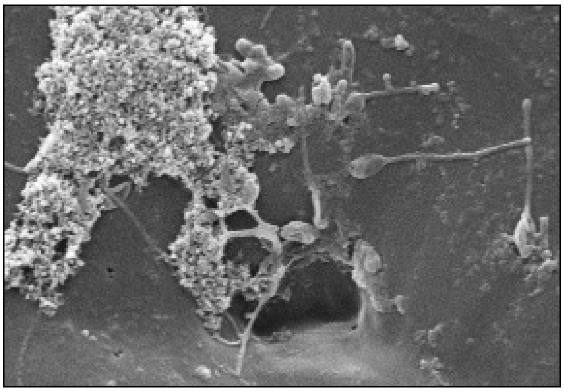

Fig. 8. SEM image of biofilm developed on surface of new pipeline made of PP
(magnification of 1 500×)

Due to a lower roughness of polyvinyl chloride and polyethylene the accumulation of the bacteria, which demonstrate greater adhesiveness than fungi, on their surfaces is easier. In such a case, the biofilm is more cohesive and less spatially diversified. The extracellular polymers developed by many species of bacteria increase their adhesiveness resulting in a closer accumulation of bacterial cells in the film; the lack of loose connections decreases the development of irregularities in the biofilm as a result of changes of the water flow rate in the water distribution system. A large number of fungi in the film contribute to the development of the film with numerous fragments whose structure is loose and a frequent detaching of whole pieces caused by the flowing medium even more diversifies the biofilm surface.

The table shows the average quantities of psychrophilic bacteria and fungi present in the biofilms developed on the materials tested.

Table

Number of microorganisms per 1cm^2 on the materials tested

Total number	PVC	PE	PB	PP
Psychrophilic bacteria	$4.4\cdot10^2$/cm^2	$9.5\cdot10^2$/cm^2	$9.7\cdot10^4$/cm^2	$18.7\cdot10^4$/cm^2
Fungi	$11\cdot10^2$/cm^2	$18.5\cdot10^2$/cm^2	$15\cdot10^4$/cm^2	$21\cdot10^4$/cm^2

The quantitative analysis of the microorganisms present on the surface of the materials tested confirmed that polyvinyl chloride demonstrates the lowest susceptibility to the overgrowth with microbial film. On the surface of polyvinyl chlorine the lowest number of both bacteria ($4.4\cdot10^2$/cm^2) and fungi ($11\cdot10^2$/cm^2) were detected. A similar number of microorganisms were observed on polyethylene. In the case of polybutylene and polypropylene, the total number of bacteria were different, namely $9.7 \cdot 10^4/cm^2$ for PB and $18.7 \cdot 10^4/cm^2$ for PP; the total number of fungi were $15 \cdot 10^4/cm^2$ for PB, $21 \cdot 10^4/cm^2$ for PP. These numbers are several times greater compared with those found on the other two materials. PP turned out to be the material with the highest number of bacteria and fungi per unit of area of surface of all the materials tested.

The effect of a metabolic activity of microorganisms on synthetic materials was assessed on the basis of the analysis of the SEM images of material samples after removing the biofilm from their surfaces with ultrasounds.

Slight damage was detected on the surface of polyvinyl chloride which is obviously connected with the smallest accumulation of microorganisms. No bacteria were found on its surface.

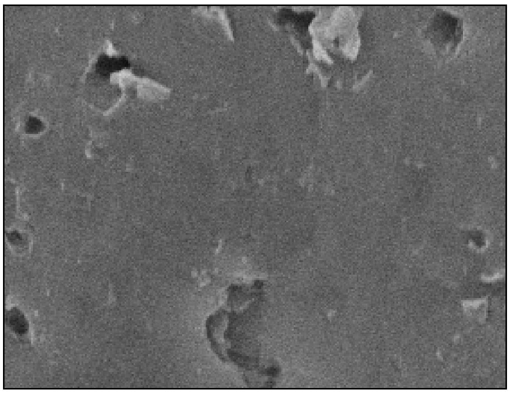

Fig. 9. SEM image of surface of new pipeline made of PVC after biofilm removal (magnification of 2 000×)

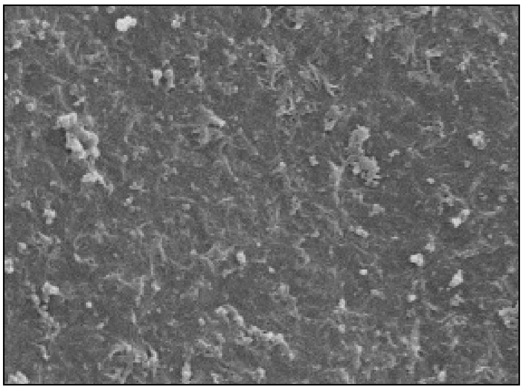

Fig. 10. SEM image of surface of new pipeline made of PE after biofilm removal (magnification of 2 000×)

Some visible changes appeared on the polyethylene surface. These were numerous irregularities that significantly increased its roughness. The number of microorganisms on this surface were close to that found on PVC, which can suggest that the substances covering the external surface of that material belong to chemical compounds easily assimilated by microorganisms.

The greatest changes were observed in the structure of polybutylene and polypropylene, since their external layers were badly damaged ("pits"). Moreover the bacterial cells present on these surfaces proved to be unremovable. Probably they were fixed to the substrate so strongly through extracellular polymers (EPS) that the ultrasonic method is not effective enough.

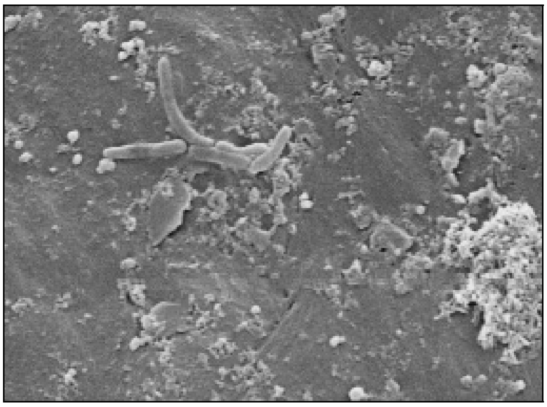

Fig. 11. SEM image of surface of new pipeline made of PB after biofilm removal (magnification of 1 800×)

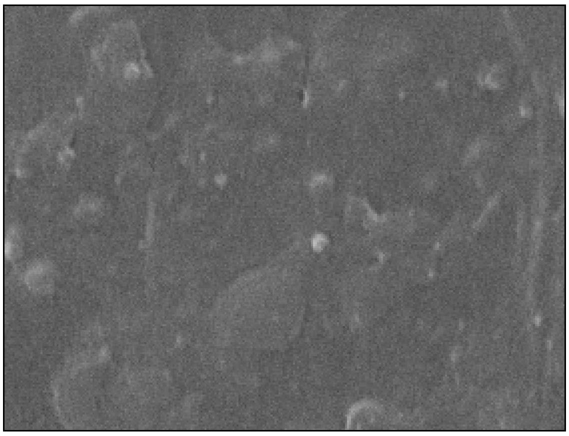

Fig. 12. SEM image of surface of new pipeline made of PP after biofilm removal (magnification of 1 800×)

4. CONCLUSIONS

• The development of biofilms on synthetic materials depends on the type of the polymer used and, to a large extent, on substrate roughness as well as the surface spatial structure.

• The greater the roughness of the substrate, the greater the percentage of the microorganisms with poorer adhesiveness.

• A spatial structure of biofilm depends on the surface of substrate on which it develops.

• The materials used for the production of pipelines might be easily assimilated by microorganisms which develop the biofilms in the tap water environment.

ACKNOWLEDGEMENT

The research performed as the "Detectors and sensors used for measuring environmental hazards – modelling and monitoring" project. Project 6, task 5, chapter 4.

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ROZWÓJ BŁONY BIOLOGICZNEJ NA POLIMERACH SYNTETYCZNYCH WYKORZYSTYWANYCH DO DYSTRYBUCJI WODY

Oceniono podatność polimerów syntetycznych wykorzystywanych do dystrybucji wody do picia na obrosty mikrobiologiczne. Oceny dokonano, określając liczebność drobnoustrojów tworzących biofilmy na poszczególnych materiałach oraz analizując zdjęcia błon biologicznych ze skaningowego mikroskopu elektronowego.