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Comparability biofilm structure on ITO sensor with forms generated on technical materials

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ABSTRACT

Spores and forms of microorganisms more resistant to disinfectants enter the water supply system as a consequence of imperfections in the treatment process. This may result in a decrease in water quality on its way to the consumers, lack of water stability, and epidemiological risk. The presence of nutrients in water contributes to the development of the dispersed state of microorganisms as well as biological membranes. A mature biological film is a complex structure comparable to an ecosystem. Regardless of the technical material used for the construction of the water supply system, there is a risk of deterioration in the mechanical and hydraulic properties of the pipes. The objective of the article was to present differences in the processes of biofilm colonisation on different materials: ITO (material used for the construction of the impedance sensor to measure single cell adhesion to pipes) and polyethylene, polypropylene, polyvinyl chloride, and polybutylene (used for the construction of the water supply system). Results indicated that the ITO material is settled in a similar way as technical material for the construction of water supply system. Therefore, it can be used as a material on sensor base to measure the biofilm development. The most susceptible to biological growth was polybutylene and polyvinyl chloride, while the more gradual grades of colonization were observed on polyethylene and polypropylene.

Keywords: AFM; Adhesion; Contact angle; DGGE; Drinking water system; Fluorescence in situ hybridization (FISH); SEM

1. Introduction

The lack of biological stability of water which is introduced into the network, leads primarily to its secondary contamination during transport to the consumer. The water that is biologically unstable creates ideal conditions for colonization of the inner surface of pipelines by microorganisms and adhesion of their products (biocorrosion). This phenomenon intensifies the electrochemical corrosion of metals, increased consumption of disinfectants, as well as the release of waste products into the water surrounding the biofilm. Biocorrosion is also a phenomenon connected with eliminating, on the one hand, the waste products causing physico-chemical corrosion and, on the other, the use of components of industrial materials as carbon and energy source by a number of heterotrophic and auto trophic bacteria. Due to the risks associated with the intensification of the biocorrosion processes the proposed project attempts to monitor the development of the biological film.

The biofilm is a complex structure of micro organisms attached to a surface, surrounded by extracellular polymers, and creating a organization of an ecosystem. This system ensures its stability, as manifested by the homeostatic ability, increased resistance to external conditions (presence of disinfectants, antibiotics), facilitating horizontal gene transfer, as well as allowing access to nutrients dissolved in the water [1].

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Biological growths are widely present in the environment and in water distribution networks. They occur as a result of supply of micro organisms to water after the cleaning process (as a consequence of the imperfections of the process) [2]. The analysis of the microbiological quality of water is based on the detection of indicator organisms, and identification of heterotrophic bacteria. So far, it has not taken into account the reservoir of micro organisms present in a form of a biological film [3,4].

A water distribution system can be treated as a bioreactor containing freely moving micro organisms, and those that settle on solid surfaces. Biological growths do not develop a uniform layer on the surface of the pipes [5]. The disconnection of single cells or groups of mature biofilm is related to the exceeding of the external shear forces [6].

Biological growths appear in the network already after several weeks of operation. The growth rate of the micro organisms depends on the characteristics of the system (approximately 76% of pipes are covered with biofilm), and the bacterial species [7,8]. Microorganisms in a compact structure are 100–1000 times more resistant to external factors [9]. In addition, multi-species biological membranes, isolated from the environment, are able to survive more than 300 times higher concentration of chlorine, and are approximately 80 times more resistant than those developed by only one species of microorganisms. This feature can be a sign of synergistic reactions that have not been discovered so far [10].

The construction of water supply systems can employ a variety of technical materials such as steel, copper, iron, polyethylene, polypropylene, polyvinylchloride, or polybutylene. Depending on the material used, the phylogenetic diversity of micro organisms is observed (PE, PVC, copper, steel) [11]. Current research shows that pipes made of polymers are colonised by microorganisms to a lower extent [7,12].

Traditional methods of detection of biofilms in water distribution networks are tedious and labour intensive. They require the presence of qualified staff, and special care during sample preparation. Results of such tests are available after a few days [13].

One of the methods of biofilm monitoring in the water supply system is impedance measurement using the sensor. Different substrates are used to create sensors and the ITO is the most common (indium tin oxide). The aim of the article was to show the similarity in technical materials colonization of the water supply system and indium oxide by using molecular biology methods.

The impedance measurement method is correct when the sensor material is non-toxic to microorganisms and with similar porosity as the materials used for the construction of water supply networks. The preliminary tests were to help show the probability bacteria adhesion of inhabiting plastics (PE, PP, PVC, PB) to the material used as the sensor base.

The proposed process of continuous measurement allows for constant monitoring of stages of the biofilm formation, permitting control over the current risks to the consumer, and taking appropriate preventive measures. The assessment of the compatibility of the sensor will enable the characterisation of micro organisms comprising the biofilm in different sources. It also allows for the determination of the interaction between micro organisms and their adhesion on plastics materials.

2. Materials and methods

Water for research was collected from 17 points from the Wroclaw Water Supply System at the consument (Fig. 1). The number of collection points was related to the size of the city district. At each point, 5 L of water was taken in

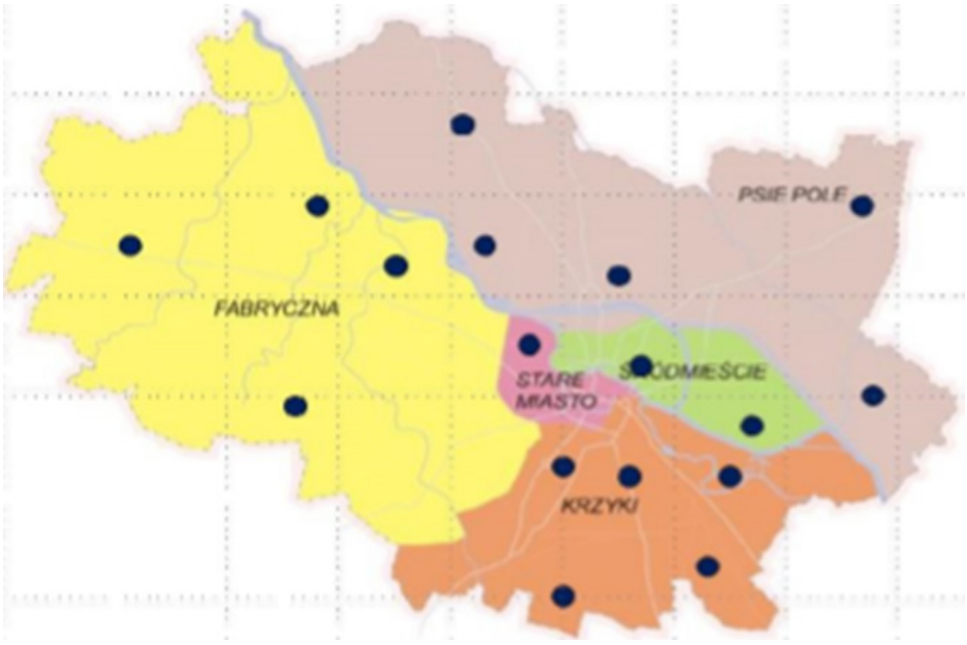

Fig. 1. Location of sampling points.

a sterile bottle and then filtered through a What man filter with a pore size of 0.2 µm. Bacteria from the filter were detached to a physiological solution (9 ml, 0.85%) using sonication bath 70 W, 35 kHz for 3 min. Subsequently, microbial culture was performed on the R2A agar medium (100 µl of sample) and after 7 d of incubation at 22°C the isolation of clean strains were done using the reduction seed method. This allowed to specify 11 strains that were repeated at each point and were considered representative of the network. That 11 strains were used for further analysis.

The first stage of such tests is to check the degree of settlement on the ITO material (selected on the sensor base) and the technical materials of the network. Therefore, model studies were carried out on the most repetitive strains, and the medium inside the reactor was synthesized water.

In a further research, the adhesion of micro organisms will be correlated with the signal coming from the impedance sensor [14].

The cultivation of the biofilm employed a bioreactor from BioSurface Technologies Corp. (Fig. 2). Experiment are performed using synthetic water (distilled sterile water and minerals such as 1.2 mmol/l $NaHCO_3$, 0.54 mmol/l $MgSO_4 \cdot 7H_2O$, 0,2 mmol/l $CaSO_4 \cdot 2H_2O$, 0,004 mmo/l K_2PO_4, 0.002 mmol/l K_2HPO_4, 0.08 mmol/l $(NH_4)_2SO_4$, 0.17 mmol/l NaCl, $36 \cdot 10^{-6}$ mmol/l $FeSO_4 \cdot 7H_2O$, 0.011 mmol/l $NaNO_3$, 0.2 mmol/l $CaCO_3$). The reactor was inoculated with a mixture of 11 bacterial strains.

After the predetermined time of culture of the biological membrane, the compatibility of the sensor (measuring the biological growth) and material forming the water distribution network made of plastic (polyethylene, polypropylene, polybutylene, and polyvinyl chloride) was verified. It was determined in order to confirm the efficacy of the method using a technique of molecular biology, namely fluorescent in situ hybridisation (FISH method) and denaturing gradient gel electrophoresis (DGGE).

2.1. Fish method

Fluorescence *in situ* hybridisation (FISH) is used to detect a given DNA sequence by means of a labelled fluorochrome oligonucleotide probe. The identification of micro organisms should apply two probes: one characteristic of the group, and a species-specific one [15].

A sample of the biofilm developed on the plastic material and sensor was fixed with 12% paraformaldehyde in buffered saline to give 4% as the final concentration of paraformaldehyde. Then it was incubated for 12 h at 4°C. The treatment protects the cells against lysis during hybridisation. Each of them was concentrated on a filter with a 0.2 µm pore diameter. After that, the filter was washed with 5 ml of sterile water and dried. In order to drain the sample, a number of alcohol dilutions (50%, 80%, 96%) was conducted, so that they become permeable to the oligonucleotides. The filters were placed on a glass slide, and covered with 1000 µl of hybridisation buffer included: 180 µl 0,9 MNaCl, 20 µl 20 mM Tris–HCl, 1 µl 0.01% SDS, 350 µl formamide, 450 µl of deionized water for BET42, GAM42 and HGC69 probes and 180 µl 0,9 MNaCl, 20 µl 20 mM Tris–HCl, 1 µl 0,01% SDS, 100 µl formamide and 700 µl of deionized water for EUB338.

Incubation was conducted at 46°C for 3 h. Due to the elevated temperature, probes bound to opposite strands of the DNA contained in the sample. Then it was incubated for 30 min with a washing solution at 48°C (NaCl changing concentration – Table 1, 20 mM Tris–HCl, 5 mM EDTA, 0,01% SDS). For visualisation, all microorganisms in a sample were stained with aqueous DAPI (4′.6–diamidyno–2–fenyloindol) solution and specific probes such as EUB338, BET42, GAM42, HGC69 compatible to specific classes of bacteria (Table 1). The observation was performed by fluorescence microscopy [15,16].

Table 1
Sequences of the probes used to FISH

Probe name	Sequence (5′ to 3′)	Formamide concentration, %	NaCl in washing buffer, mM
EUB338	CTGCCTCCCGTAGGAG	20	0.9
BET42	CCTTCCCACTTCGTT	35	80
GAM42	GCCTTCCCACATCGT	35	80
HGC69	ATAGTTACCACCACCGCCG	35	

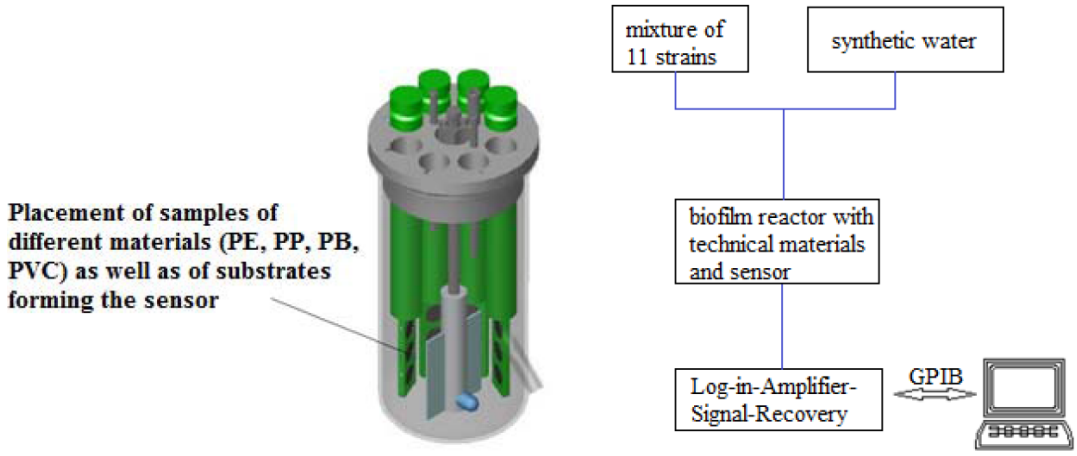

Fig. 2. Schematic of the reactor for biofilm cultivation and diagram of the measuring system.

To control non-specific binding of EUB 338, probe NON 338 was used. Hybridisation with NON 338 gave no signals despite background fluorescence of the tissue.

The material formed on the pipe was analyzed in a scanning electron microscope (SEM). For this purpose, samples were fixed with 2.5% glutaraldehyde, than dehydrated through a graded series of ethanol (30%, 50%, 70% —once for 10 min at each step), and covered with gold in Scancoat machine (Oxford). The images were taken with a Leo Zeiss 435 Vp (Oberkohen) scanning microscope.

During the studies, specially constructed test sensors made in the photo lithography process was used. The sensor placed in the reactor was connected to the measuring apparatus (Lock–in–Amplifier Model 7280), which continuously recorded changes in impedance (conductance change symbolizes the process of adhesion of microorganisms). The entire measurement is automated and the control of individual stages is carried out by means of an application written in LabVIEW. The program allows measurement of impedance components, and its graphical presentation.

2.2. DGGE (Denaturing gradient gel electrophoresis)

The development of molecular techniques permitted revealing variability in bacterial genetic material. One of the techniques is denaturing gel gradient electrophoresis (DGGE). During the electrophoresis of two-strand DNA in polyacrylamide gel with increasing gradient of the denaturing agent (urea, formamide), a part of DNA fragments is subject to division into single strands at a lower, and the other part at a higher concentration of formamide. The denaturation process depends on the DNA structure (type of alkali and length). Single-strand DNA fragments are characterised by reduced electrophoretic mobility after reaching its denaturation point. This permits separation of fragments of DNA particles which in the two-strand form have similar mobility at different melting properties. The separated bars can be cut out, again subject to amplification, and sequenced, or subject to blotting and hybridised with a specific molecular probe. The fingerprinting method related to the conditions of melting of amplified DNA chains allows for the observation of both spatial and temporal changes in the structure of the community of micro organisms, and detection of the dominant strains in the sample [17,18].

2.3. Isolation of bacterial genome DNA

The isolation of bacterial DNA was performed based on the universal equipment for isolation of genome DNA from different materials Genomic mini by A&A biotechnology.

The technical materials and sensor on which the biofilm developed as a result of conducting a reactor culture were placed in sterile test tubes with a 0.85% physiological solution (0.85 g NaCl dissolved in 1 l distilled water) was used for shaking, and shaken for 24 h at room temperature. Next, the residue of the solution was scraped by means of a sterile scalpel. The sample was centrifuged for 10 min. at 1151 rad/s. Then, the supernatant was removed, and the sediment was thoroughly suspended in 100 µl of Tris buffer. Due to the mixture of bacteria (both Gram+ and Gram-), relevant enzymes destroying cell walls of Gram+ bacteria were applied: 5 µl of mutanolysin (10 U/µl) and 10 µl of lysozyme (10 mg/ml). The sample was stirred and incubated for 20 min. at a temperature of 50°C. The procedure followed the manufacturer's protocol.

200 µl of lysis solution LT and 20 µl of Proteinase K was added to the sediment with digestive enzymes. The samples were mixed by inverting the tube, and then incubated for 20 min. each at a temperature of 37 and 70°C. The samples were vortexed for 30 s, and then centrifuged for 3 min at 1571 rad/s. Supernatants were deposited on mini columns included in the set, and centrifuged for one minute at 1571 rad/s. Then, 500 µl of flushing solution A1 was deposited on the mini columns and subject to centrifuging. The procedure was repeated twice. At the final stage, isolated DNA was washed from the mini columns using 100 µl of Tris buffer heated to 70°C to sterile Eppendorf test tubes, and stored at a temperature of –20°C until further analyses.

For the purpose of purity control, the reaction mixture was subject to agarose electrophoresis in 0.8% gel with addition of 4 µl of ethidium bromite (0.5 µl/ml).

2.4. Amplification of region 16S of rDNA

Each 50 µl of the PCR solution contained: 25 µl 2×buffer for PCR reaction (final concentration of $MgCl_2$ 1.75 mM), 10 µl 2 mM dNTPs, 3 µl of each of the starters (final concentration in total volume 0.3 µM), 1 µl Taq of polymerase, 2 µl of matrix DNA (concentration of approximately 200 ng), and deionised water free from DNA and RNA. The reaction was conducted in a Master cycler pro by Eppendorf [19]. Universal starters used for the PCR reaction had the following sequence: 27F 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R 5′-TACCTTGTTACGACTT-3′, resulting in a product with a length of 1500 pz [20].

For the purpose of purity control, the reaction mixture was subject to agarose electrophoresis in 0.8% gel with addition of 4 µl of ethidium bromide (SIGMA). The evaluation of the length of the resulting products employed a marker with a range from 100 to 3000 pz (DNA Gdańsk).The conditions of the process are presented in Table 2.

2.5. Conditions of amplification of region V3-V5

Region V3-V5 16S rDNA was amplified with the application of starters 341F 5′-CCTACGGGAGGCAGCAGCCG*-3′ [*GC clamp CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGG] and 907R 5′- CCGTCAATTCCTTTGAGTTT- 3′, providing products with a size of 440 pairs

Table 2
Conditions of PCR reaction for 16S of rDNA

Stage	Temperature, °C	Time, s	Number of cycles
Preliminary denaturation	94	300	1
Denaturation	94	30	30
Hybridisation	50	60	
Elongation	72	50	
Final elongation	72	300	1

of alkalis [21]. The PCR reaction mixture contained 25 µl of polymerase 2 × PCR TaqNova-RED Master Mix (DNA Gdańsk), 1 µl of each starter (10 µM), 1ng of matrix DNA. It was supplemented to 50 µl with water free from DNA and RNA. The PCR reaction was conducted in a Master cycler pro by Eppendorf. For the purpose of purity control, the reaction mixture was subject to agarose electrophoresis in 0.8% gel with addition of 4 µl of ethidium bromide (SIGMA). The agarose gel was prepared based on buffer 1x TBE (Tris-HCl, boric acid, 0.5 M EDTA with pH = 8). The electrophoresis occurred at a voltage of 90 V for 90 min in a device APELEX with power supply PS503XL . The conditions of the process are presented in Table 3.

2.6. Conditions of amplification of region V6-V8

Region V6-V8 16S of rDNA was amplified with the application of starters 968 5′-AACGCGAAGAACCTTAC-3′ and 1401R 5′-CGGTGTGTACAAGGCCCGGGAACG-3′, providing products with a size of 440 pairs of alkalis [23]. The PCR reaction mixture contained 25 µl of polymerase 2×PCR TaqNova-RED Master Mix (DNA Gdańsk), 1 µl of each starter (10 µM), 1 ng matrix DNA. It was supplemented to 50 µl with water free from DNA and RNA. The PCR reaction was conducted in a Master cycler pro by Eppendorf. For the purpose of purity control, the reaction mixture was subject to agarose electrophoresis in 0.8% gel with addition of 4 µl of ethidium bromide (SIGMA). The agarose gel was prepared based on buffer 1×TBE (Tris-HCl, boric acid, 0.5 M EDTA with pH = 8). The electrophoresis occurred at a voltage of 90 V for 90 min in a device APELEX with power supply PS503XL. The conditions of the process are presented in Table 4.

The product of the PCR reaction with starters amplifying regions V3-V5 and V6-V8 16S of rDNA was mixed with ballast (1 µl of buffer per 5 µl of volume of the sample). The electrophoresis was conducted for 16 h at a voltage of 75 V in a device by C.B.S. Scientific in 8% gel. The range of the denaturing agent amounted to 30–70%. The polyacrylamide gel was transferred to 250 ml of buffer TAE (1X) and 25 µl of ethidium bromide with a concentration of 10 mg/ml. Staining was conducted for 15 min. Then, the gel was transferred to a clean solution and discoloured for 20 min. Photographs were taken by means of a transluminator, and analysed [25].

2.7. Microscopic observations

The material developed on the pipe was analysed in a scanning electron microscope (SEM). First it was fixed with 2.5% glutaraldehyde, than the samples were dehydrated through a graded series of ethanol (30%, 50%, 70% — once for 10 min at each step), after that they were coated with gold using Sputter coater and than observed.

For this purpose, samples were covered with gold in a Scancoat machine (Oxford). The images were taken by means of a Leo Zeiss 435 Vp (Oberkohen) scanning microscope.

The research applied specially constructed test sensors made in the photo lithography process. The sensor placed in the reactor was connected to the measuring apparatus (Lock–in–Amplifier Model 7280) which continuously recorded changes in impedance (conductance change symbolises the process of adhesion of micro organisms). The entire measurement is automated, and the control of individual stages is carried out by means of an application written in LabVIEW. The program allows measurement of impedance components, and its graphic presentation.

Water contact angle measurements were made using surface free energy evaluation system (Advex Instruments s.r.o.) with distilled water as a wetting agent. The surface was defatted using acetone. Single drop of distilled water was put on sample's surface at least 5 times. According to the experimental regime, volume of the deposited drop remained unchanged during the test (drop was freely deposited from the same height). Measured contact angle was averaged, extreme values (differenting more than 20% from averaged value) were ommited. AFM was provided using Veeco Nanoman VS Micro scope working in tapping mode.

Table 3
Conditions of amplification V3-V5

Stage	Temperature, °C	Time, s	Number of cycles
Preliminary denaturation	94	300	1
Denaturation	94	40	10
Hybridisation	65 to 56 (–1°C per cycle)	60	
Elongation	72	45	
Denaturation	94	40	25
Hybridisation	56	60	
Elongation	72	45	
Final elongation	72	600	1

Table 4
Conditions of amplification of V6-V8

Stage	Temperature, °C	Time, s	Number of cycles
Preliminary denaturation	95	180	1
Denaturation	95	30	20
Hybridisation	65 to 56 (–0.5°C per cycle)	30	
Elongation	72	60	
Denaturation	95	30	25
Hybridisation	56	30	
Elongation	72	60	
Final elongation	72	300	1

3. Results and discussion

Impedance measurement was conducted for 30 d under continuous stirring, then flow conditions were provided. According to the literature, it is sufficient time to produce a young biofilm structure.

Fluorescent analysis of the sensor surface showed the presence of living cells (2A). The largest group was class

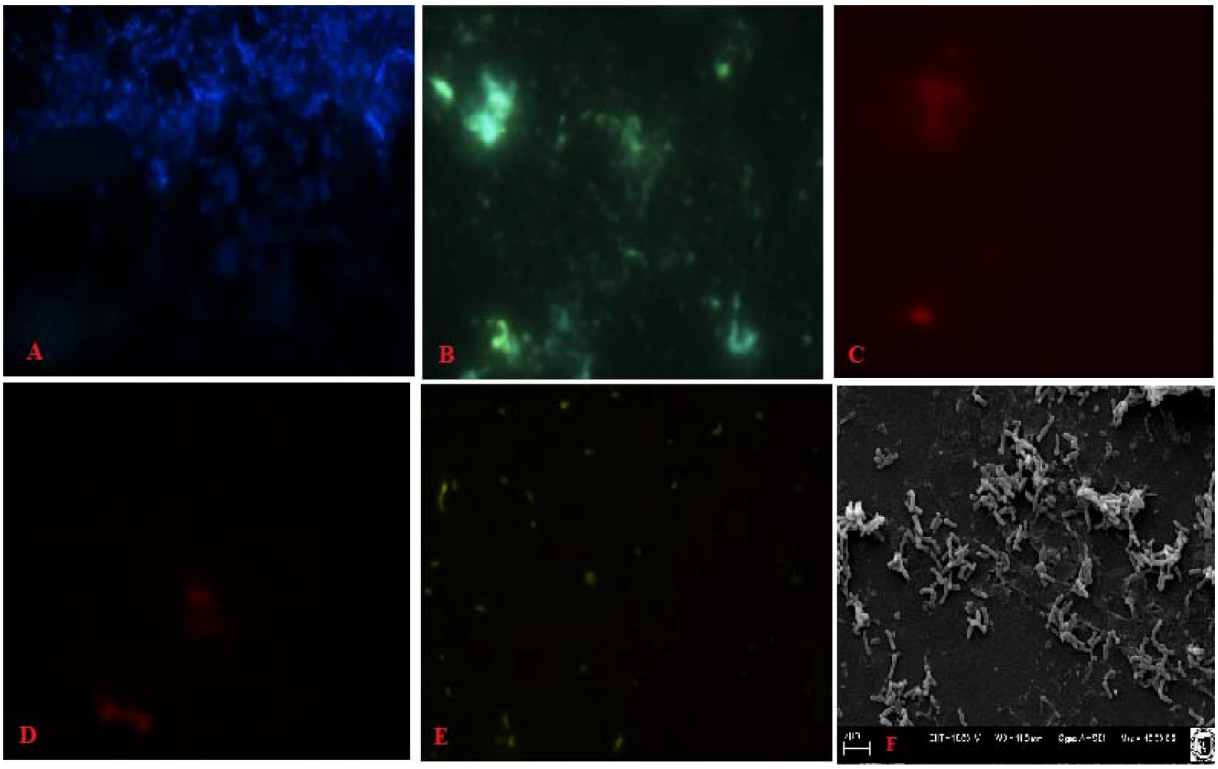

Fig. 3. Visualisation of bacteria in fluorescent microscopy after test measurement on sensor surface (lighter places – presence of bacteria); A – all cells – blue colour, B – *Eubacteria* class – green colour, C – *Betaproteobacteria* class – red colour, D – *Gammaproteobacteria* class – green colour, E – *Actinobacteridae* class – red colour, F – SEM micro graphs of the biofilm on sensor (1 µm).

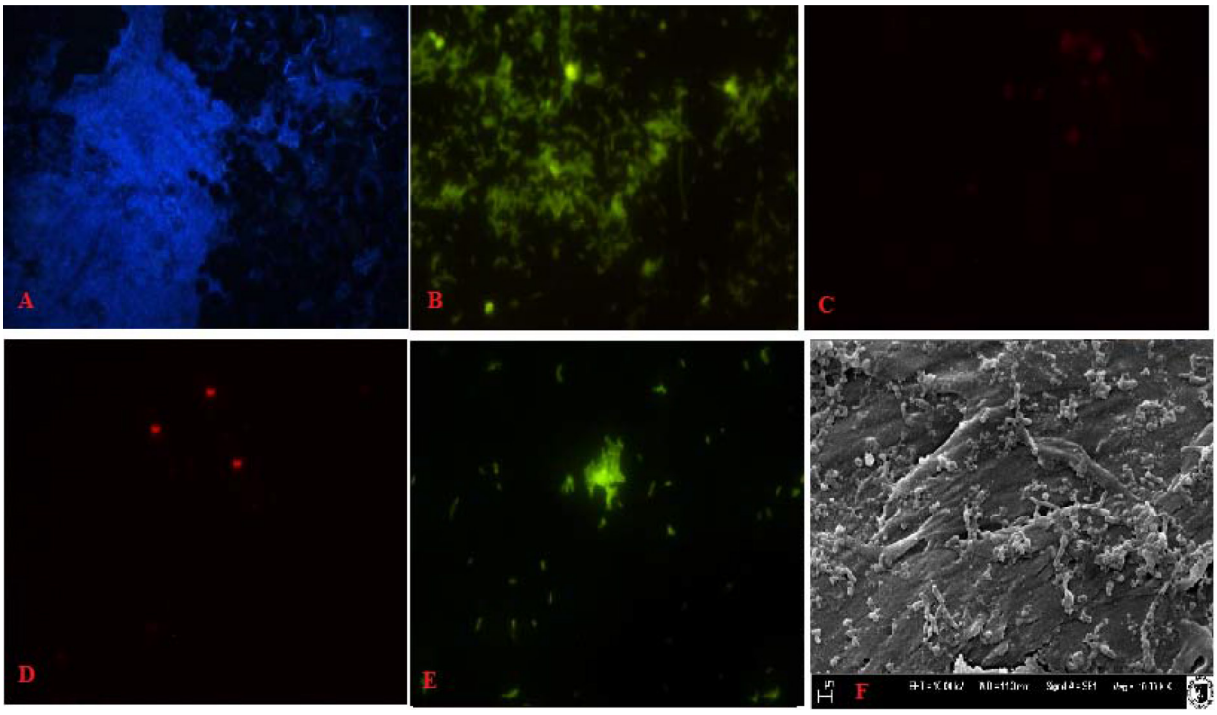

Fig. 4. Visualisation of bacteria in fluorescent microscopy after test measurement on a fragment of water distribution network made of polypropylene (lighter places – presence of bacteria); A – all cells – blue colour, B – *Eubacteria* class – green colour, C – *Betaproteobacteria* class – red colour, D – *Gammaproteobacteria* class – green colour, E – *Actinobacteridae* class – red colour, F– SEM micro graphs of the biofilm on polypropylene.

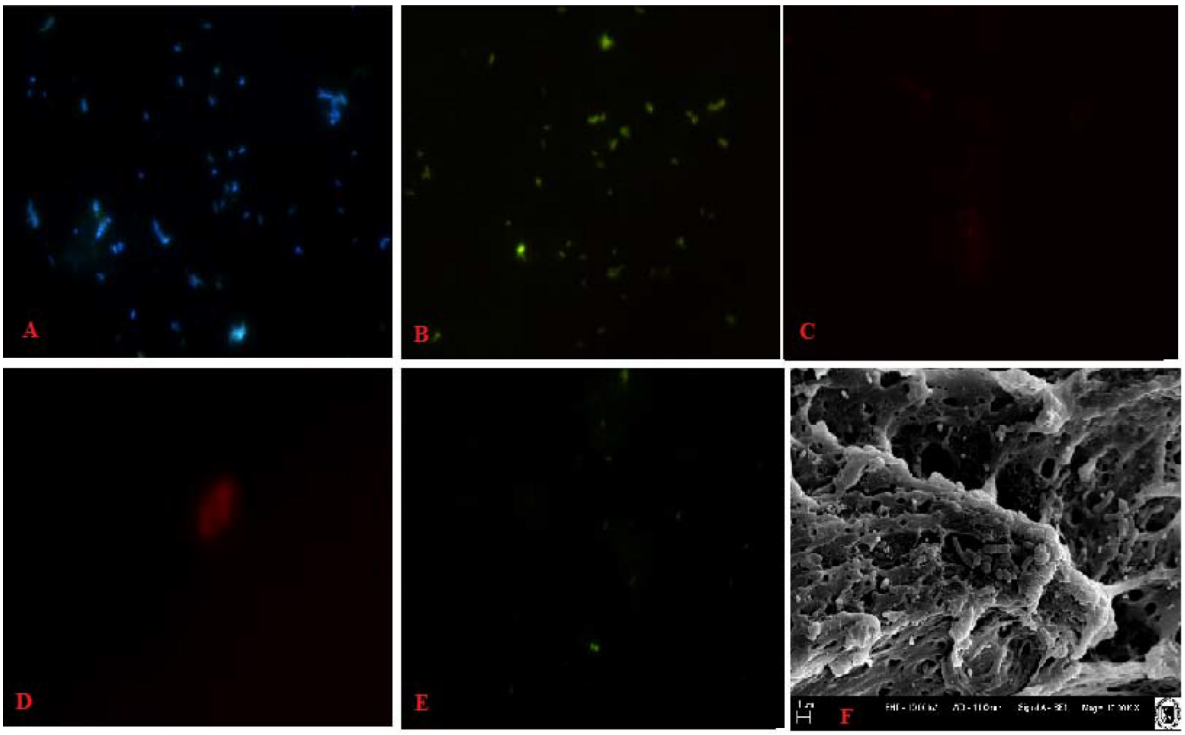

Fig. 5. Visualisation of bacteria in fluorescent microscopy after test measurement on a fragment of water distribution network made of polyethylene (lighter places – presence of bacteria); A – all cells – blue colour, B – *Eubacteria* class – green colour, C – *Betaproteobacteria* class – red colour, D – *Gammaproteobacteria* class – green colour, E –*Actinobacteridae* class – red colour, F – SEM micrographs of the biofilm on polyetylene.

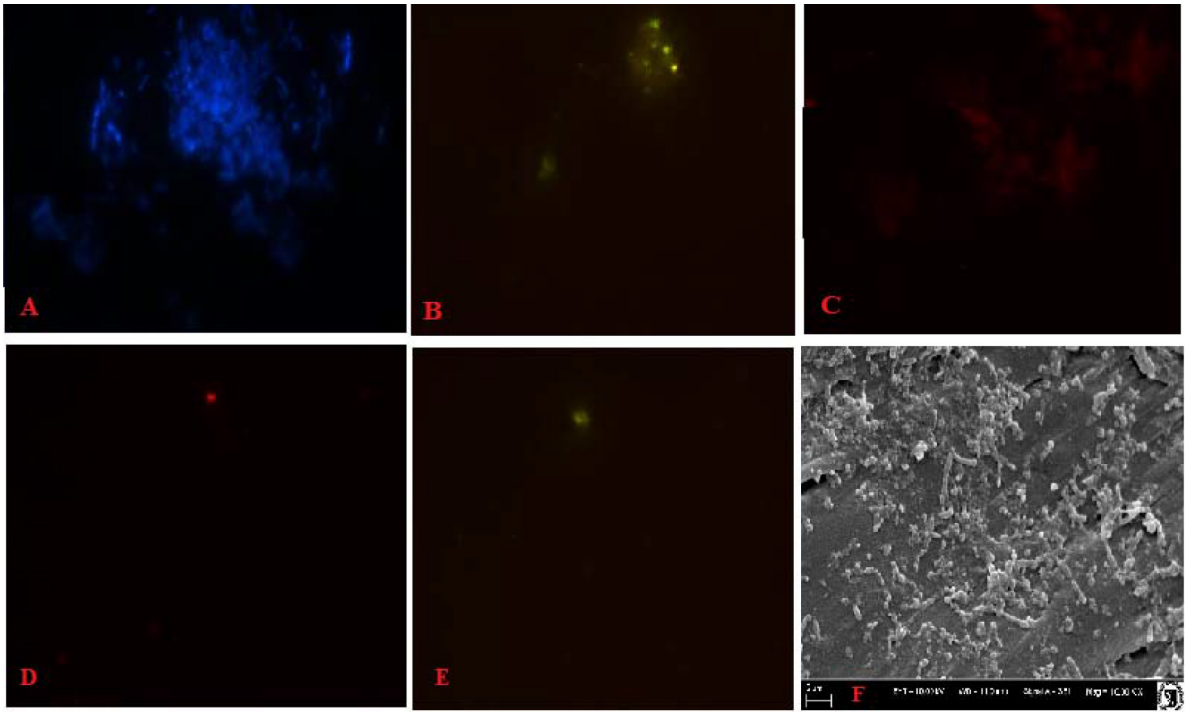

Fig. 6. Visualisation of bacteria in fluorescent microscopy after test measurement on a fragment of water distribution network made of polyvinyl chloride (lighter places – presence of bacteria); A – all cells – blue colour, B – *Eubacteria* class – green colour, C – *Betaproteobacteria* class – red colour, D – *Gammaproteobacteria* class – green colour, E – *Actinobacteridae* class – red colour, F – SEM micro graphs of the biofilm on polyvinyl chloride.

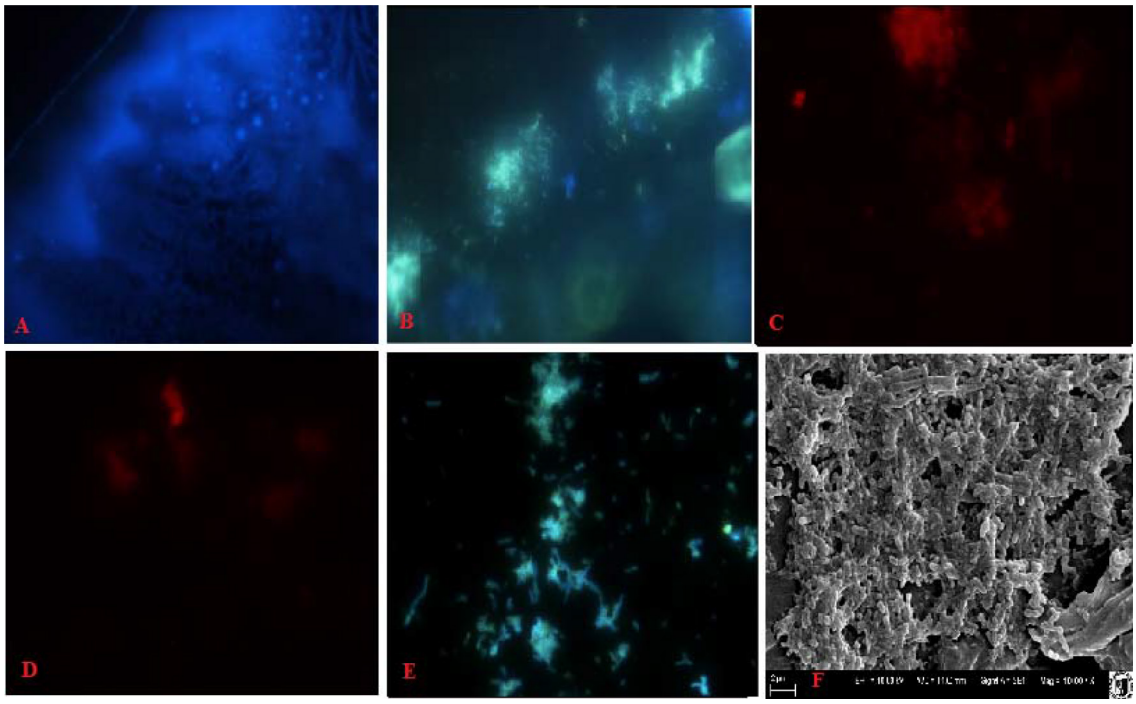

Fig. 7. Visualisation of bacteria in fluorescent microscopy after test measurement on a fragment of water distribution network made of polybutylene (lighter places – presence of bacteria); A – all cells – blue colour, B – *Eubacteria* class – green colour, C – *Betaproteobacteria* class – red colour, D – *Gammaproteobacteria* class – green colour, E –*Actinobacteridae* class – red colour, F – SEM micro graphs of the biofilm on polybutylene.

Eubacteria (2B). The predominance of class *Beta*–over *Gammaproteobacteria* (2C, 2D) was also determined. SEM analysis confirmed the presence of bacterial cells on the sensor surface. Single cells were observed in larger groups not producing extracellular polymers (2F). It was made on the glass which has an ideal surface, even though the bacteria was attached on it.

FISH analysis of the polypropylene surface showed that the majority of micro organisms hybridise with probes having a sequence characteristic of *Eubacteria* (3B). Immersion microscopic observation showed prevalence of class *Gammaproteobacteria* over *Betaproteobacteria* (3C,D). Class *Actinobacteridae* bacteria were present in large quantities in the groups (3E). SEM analysis showed the development of a structure with large groups of cells. Groups of micro organisms were surrounded by polymers (3F).

Also polyethylene was characterised by low efficacy of probe hybridisation for *Eubacteria* (4B). The samples included a comparable amount of micro organisms from the *Gamma–*, *Beta–* and *Actinobacteridae* group (4C,D,E). The polyethylene surface on the images obtained by SEM analysis showed low colonisation by micro organisms. There was no continuous structure but only a group of micro colonies without EPS (4F).

The analysis results for the surface of a pipe made of polyvinyl chloride showed extremely low hybridisation with *Eubacteria* probe (5B). The preparations show a significant predominance of *Betaproteobacteria* over *Gammaproteobacteria* and *Actinobacteridae* (5C,D,E).

The highest total amount of bacteria was observed on polyvinyl chloride and polybutylene. Polyvinyl chloride and polybutylene are materials on which biological membranes have the most complex structure in SEM analysis with visible channels between bacterial groups (5F, 6F).

Abiotic or hydrophobic abiotic surfaces (positively charged) are generally better attached by micro organisms than hydrophilic or negatively charged surfaces.

Such areas include polyvinyl chloride or polyethylene. Most bacteria are negatively charged, and many surface structures, proteins, or lipids increase their hydrophobicity. Strong hydrophobic properties of the surface affect auto aggregation of bacterial cells and indicate a higher degree of adhesion to hydrophilic surfaces.

This study confirmed the above only for the polyvinyl chloride surface. Polyethylene proved to be the least populated material. Pipes made of plastic are believed to be characterised by low surface roughness. This can minimise the formation of the biological membrane. Such composites, however, can be the source of nutritious substance for micro organisms. Chemical compounds used in the process of their production, such as hardeners, fixatives, or stabilisers, can be washed out and become a potential substrate for microbes, and stimulate their growth [26].

The presence of biological membrane increases the maintenance costs of the drinking water distribution network [27]. According to Sarro [28], bacteria in the biological membrane belong to five phylogenetically different classes: *Alfaproteo bacteria, Betaproteo bacteria, Gammaproteo bacteria, Actino bacteridae,* and *Firmicutes.* The strains considered as reference were included in these classes.

Bacterial adhesion to PVC is closely related to cell hydrophoby [29]. In the case of biological growth on polyvinyl chloride, a similar tendency was observed by [30],

involving significant domination of class *Betaproteo bacteria* over *Gammaproteo baceria*.

The study showed that the biological membrane developed on the polypropylene surface mostly consisted of class *Gammaproteo bacteria*. The group includes pathogenic and potentially pathogenic bacteria. None of the 11 strains used for the tests showed pathogenic character, which does not confirm the results obtained by Mathieu [31]. According to Bellon [32], polypropylene is easily colonised by micro organisms. This effect can be caused by its fibrous structure supporting adhesion.

Henne et al. [33] observed that the structure of the biological membrane depends on the type of material on which it develops. Yu [34] also confirms this theory. Moreover, the authors show that polyethylene and polybutylene are colonised as efficiently as stainless steel. In addition, pipelines made of plastics can allow the development of compounds. Some of them might be used as nutrients by micro organisms.

Research by Różej [35] revealed that PVC is different from other plastics materials. The large number of bacteria accumulated on the pipe exceeded the amount of those accumulated on the polyethylene surface. The results were also confirmed by the FISH method, where the largest number of bacteria was observed on the PCV and polybutylene surface.

According to research by Waines [36], no material used for the construction of the water distribution system is free from micro organisms. This is confirmed by the results of this study.

3.1. Biodiversity of microorganisms –DGGE

As a result of electrophoresis of amplified DNA fragments of region V3-V5 form the biofilm collected on different technical materials made of plastics, eight bars were obtained on polypropylene, seven bars on polyethylene, 8 bar on polyvinyl chloride, and seven bars on polybutylene. In the case of analysis of the biological membrane developed on the sensor, eight bars were obtained. 11 bar were obtained in none of the cases, number of bacterial strains introduced to the applied reactor.

The analysis of the amplified region V6-V8 16S DNA for the mixture of 11 strains used in the experiment showed that the sensor built on ITO substrate was characterised by high adjustment to the used strains (10 bar). The comparison of technical materials made of plastics used in the construction of the water supply network showed the lowest degree of adhesion to the used strains for polyethylene –7 bar (which confirms the performed FISH analyses). The highest number of bars was observed on PVC material. This suggests a high degree of adhesion of microorganisms to this technical substrate.

3.2. Contact angle

Contact angle measured on all surfaces increased in order: PVC<PB<PP<PE (refer to Fig. 9), reflecting increasing hydrophobic character of subsequent samples. That observation is consistent with theoretical divagations concerning chemical composition of the polymer molecule and stays in good agreement with earlier divagations concerning probability of bacterial growth on subsequent polymer samples. Colonization of pipes made of different polymers also increases in the same order as hydrophobicity. The differences among samples are lower than estimated error (measured as standard deviation) but tendency is obvious and too remarkable to be neglected. It has to be mentioned that contact angle reflects both chemical and physical character of the surface (refer to Fig. 10). As the influence of the chemical compositions to measured contact angle is trivial the roughness (physical character) is not an easy factor to be determined. Porosity may increase the adhesion force among bacteria and surface. It seems that in analyzed systems porosity decreased as the hydrophobicity increased being thus an additional factor in the living cell – solid phase adhesion interaction.

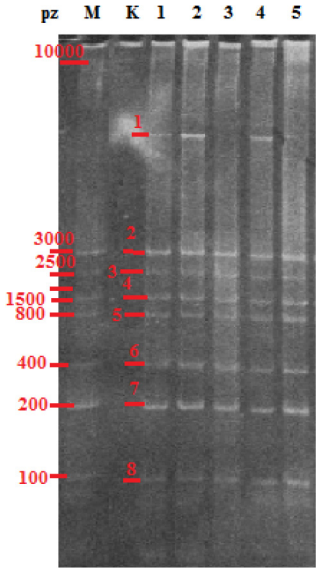

Fig. 8. DGGE of region V3-V5 of biofilm from the surface: 1-SEN, 2-PP, 3-PE, 4- PVC, 5-PB, M-marker of size, 10 kpz.

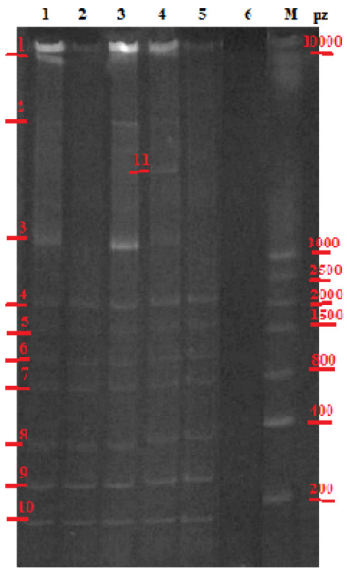

Fig. 9. DGGE of region V6-V8 of the biofilm from the surface: 1-SEN, 2-PE, 3-PP, 4- PVC, 5-PB, 6- K, M-marker of size, 10 kpz.

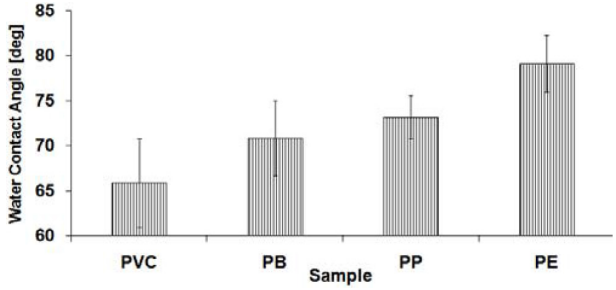

Fig. 10. Water contact angle measured for all samples.

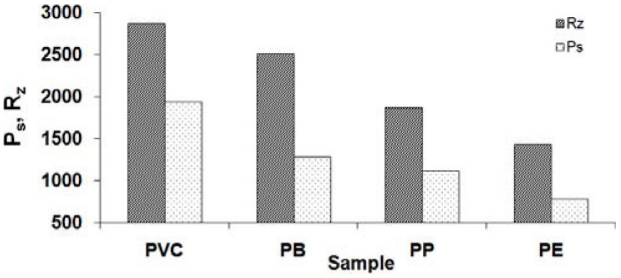

Fig. 11. Surface roughness parameters in measured samples.

4. Conclusion

Water intended for consumption is not completely free of microorganisms, which is a consequence of the biological membranes presence in the water network

Because of the awareness of the imperfections in the water intended to the consumption, fast and effective methods of controlling the microorganisms adhesion are sought, for example using sensors.

The test sensor was made on glass, which is considered as perfectly sleek, while plastics are materials with a very small surface roughness, in spite of that on both surfaces there was a biological membrane

Porosity and hydrophilic character increases probability of colonization by bacterial strains; increasing in order: PE<PP<PB<PVC.

Qualitative and quantitative microbiological analyses are related to the long detection time, and the obtained results do not clearly reflect the sanitary quality of water; therefore, the aim is to create a probe for biofilm monitoring, which attached to the inner surface of the tube would enable continuous measurement, giving results in real time, which in turn will result in a low cost of measurement and its appropriate sensitivity.

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