Growth of ZnO nanowires on polypropylene membrane surface—Characterization and reactivity

Marta Bojarska\(^a\), Bartosz Nowak\(^a\), Jarosław Skowroński\(^c\), Wojciech Piątkiewicz\(^c\),\(^d\), Leon Gradoń\(^d\)

\(^a\) Warsaw University of Technology, Faculty of Chemical and Process Engineering, Waryńskiego 1, 00-645 Warsaw, Poland
\(^b\) Lehrstuhl für Technische Chemie II, Universität Duisburg-Essen, Essen 45117, Germany
\(^c\) Institute for Sustainable Technologies—National Research Institute, Pułaskiego 6/10, 26-600 Radom, Poland
\(^d\) PolymemTech Sp. z o.o., al. Niepodległości 118/90, 02-577 Warsaw, Poland

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Need for a new membrane is clearly visible in recent studies, mostly due to the fouling phenomenon. Authors, focused on problem of biofouling caused by microorganisms that are present in water environment. An attempt to form a new membrane with zinc oxide (ZnO) nanowires was made; where plasma treatment was used as a first step of modification followed by chemical bath deposition. Such membrane will exhibit additional reactive properties. ZnO, because of its antibacterial and photocatalytic properties, is more and more often used in commercial applications. The authors used SEM imaging, measurement of the contact angle, XRD and the FT–IR analysis for membrane characterization. Amount of ZnO deposited on membrane surface was also investigated by dithizone method. Photocatalytic properties of such membranes were examined through methylene blue and humic acid degradation in laboratory scale modules with LEDs as either: wide range white or UV light source. Antibacterial and antifouling properties of polypropylene membranes modified with ZnO nanowires were examined through a series of tests involving microorganisms: model gram-positive and –negative bacteria. The obtained results showed that it is possible to modify the membrane surface in such a way, that additional reactive properties will be given. Thus, not only did the membrane become a physical barrier, but also turned out to be a reactive one.

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1. Introduction

With the increasing world population, problems concerning water pollution grew significantly in last few years and that is why the use of disinfectants is so important [1–3]. Pathogenic microorganisms such as: Staphylococcus, Enterococcus, or Streptococcus, present in natural water and wastewaters, are a serious threat to public health [4,5]. Membranes can be used for: bacteria, macro-
molecules, organic compounds, salts or even viruses removal. In the last couple of years membranes become more widespread because of their reliability and ease of use [2,6–9]. Particularly, the microfiltration turned out to be less costly than other purification methods. Microfiltration is often used as a pre-filtration process prior to nanofiltration, reverse osmosis, or as a key element in bioreactors [6,8]. Despite significant potential of microfiltration and membranes, problem of organic fouling and biofouling lowers their efficiency [4,6,7]. Also large amounts of natural organic matter (NOM) in e.g. surface waters make organic fouling inevitable. Fouling is mostly caused by formation of a filtration cake on the membrane surface, which blocks pores or reduce their size [10,11]. Biofouling or microbiological fouling is considered to be far worse because of microorganisms ability to multiply and surface colonization [6–8]. The moment the microorganism sticks to the surface a complicated, multistage process leading to biofilm formation begins. At the same time extracellular polymeric substances (EPS) such as polysaccharides and other organic substances (e.g. proteins

Abbreviation: CBD, chemical bath deposition; CIP, cleaning in place; COD, chemical oxygen demand; EPS, extracellular polymeric substances; HA, humic acid; HMTA, hexamethylenetetramine; HT, hydrothermal growth; LB, Lysogeny Broth; M9, minimal broth; MB, methylene blue; NOM, natural organic matter; OD, optical density; ROS, reactive oxygen species.

* Corresponding author at: Warsaw University of Technology, Faculty of Chemical and Process Engineering, Waryńskiego 1, 00-645 Warsaw, Poland.
E-mail addresses: m.bojarska@chip.pw.edu.pl (M. Bojarska),
novakbartosz@gmail.com (B. Nowak), jaroslaw.skowronski@ifw.radom.pl
(J. Skowronski), w.piatkiewicz@polymemtech.com (W. Piątkiewicz),
I.gradon@chip.pw.edu.pl (L. Gradoń).

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or nucleic acids) are released [7,12]. Biofilm formation presented in Fig. 1 [6,11–13] includes the following stages:

- bacteria in planktonic form (1);
- attachment of bacteria (2);
- release of EPS (3);
- maturation of biofilm (4);
- detachment (release) of bacteria (5).

In case of membrane filtration there are several ways to reduce the problem of fouling/biofouling, like: cross-flow filtration, stream disinfection (pasteurization, UV radiation, potassium permanganate, chlorates, etc.), back puls, or cleaning in place (CIP) [10]. Total recovery of the filtration stream is impossible due to irreversible adhesion of some biofilm components to the membrane surface. First stages of biofilm formation require a membrane–pollutant interaction, thus it is crucial to interrupt this process at this stage. Membrane surface modification can reduce the problem of fouling [6,9,10,12,14,15]. That is why there is a need for new membranes, with photocatalytic or antibacterial properties. Those properties may reduce the problem of both organic fouling and biofouling [4]. There are two types of membranes with nanoparticles in their structure: membranes with a thin layer of nanoparticles on their surface or mix matrix membranes, which have nanoparticles in all their volume [16]. Due to surface nature of the fouling phenomena, authors decided on the first approach.

ZnO is common in natural world and it is environmentally friendly [17–23]. Zinc oxide is a semiconductor with of wide band gap of 3.37 eV and high bonding energy (60 meV). ZnO can absorb UV radiation with the wavelength not exceeding 385 nm, which is connected with band gap, and visible light with the peak between 450 and 750 nm, which can be associated with the defect in the zinc oxide crystal [23]. In case of this research, absorption peaks related to crystal defects are desired. Jones et al. [5] reported in their work that 4% or less UV radiation present in fluorescent light is sufficient for the activation of zinc oxide. In their work they also claim that visible light, present in the laboratory, is sufficient for activation of ZnO [5]. Because of high electron mobility (better than TiO2) and ease of fabrication, ZnO-based nanostructures are of interest to the photocatalysis [24]. ZnO doping by Graphene Oxide [25], Sn [26], Ag [27] or Ni [28], can even enhance photocatalytic activity of ZnO in visible light, or reduce the e−–h+ pair recombination. Thanks to its photocatalytic properties, zinc oxide can be used for prevention of organic fouling. Photocatalysis is process activated by light, where on the semiconductor an electron–hole pair is generated (Fig. 2) [21,29,30].

Pair e−–h+ participates in redox reactions with elements adsorbed on the surface of catalyst. The hole can oxidize water to a hydroxyl radical, which then can initiate reactions leading to oxidation of organic compounds. Electron can be transferred to an electron acceptor, e.g. oxygen or metal ion, which has more positive redox potential than the band gap of the photocatalyst (reduction of metal ion to metal and deposition on photocatalyst surface) [31]. Photocatalysis is used for decolorization, degradation of dyes, chemical oxygen demand (COD) reduction, mineralization of toxic organic components, removal of heavy metal ions, degradation of harmful fungicides, herbicides, pesticides, water purification and disinfection [29,31,32].

Zinc oxide also exhibits high toxic properties against both gram–positive and –negative bacteria [2,33–37]. ZnO can also destroy spores [38]. It exhibits anti–fungal properties, for which it has been known since the 1990s, e.g. against Aspergillus brasiiliensis—aggressive and widely occurring fungus [36,39]. Mechanism of antibacterial activity of zinc oxide is not well known. It is assumed, that it is mainly based on the formation of reactive oxygen species (ROS) from water and oxygen that disrupt the integrity of bacterial membrane, oxidize proteins and damage DNA or inhibit replication of DNA. The role of zinc ions is not clear, it is suggested that ions bind with the membrane of microorganisms, and extend the lag phase during the growth of bacteria; they also can damage DNA, disrupt enzymes, interrupt transmembrane electron transport and cause protein denaturation (Fig. 3) [2,33,36,37,39,40,41]. In addition, microorganisms that have been deposited on wurtzite modified membrane surface may be mechanically damaged by the nanowires. The cell is suspended on nanowires and the cell wall breaks due to the resulting mechanical stress [28,42]. Authors focused on ZnO as the modification material, due to described above few antibacterial mechanisms. In contrast, more widely used titanium dioxide antibacterial mechanism, is mainly associated with ROS formation and oxidative stress [43].

Zinc oxide nanoparticles are toxic to e.g.: Escherichia coli [35,37,39], Bacillus subtilis [35,37,39], Pseudomonas fluorescens [39], Pseudomonas aeruginosa [37], Staphylococcus epidermis [5,37], Staphylococcus agalactiae [39], Staphylococcus aureus [5,37,39,44], Candida albican [37], Enterococcus faecalis [37], Streptococcus pyogenes [5] or Streptococcus mutans [45]. There are also studies on the ZnO toxicity with respect to their potential environmental effects (influence of ZnO on algae—Chlorella sp.) [46]. Still, there is no clear
answer if zinc oxide nanoparticles are also toxic to mammalian cells or not. However immobilization of zinc oxide nanowires on membrane surface might reduce its eventual hazardous properties. Broad spectrum of microorganisms that zinc oxide nanoparticles are toxic to, is one of the main reasons why this nanomaterial was chosen over others (like very popular titanium dioxide) for membrane modification.

Zinc oxide can be present in various crystalline forms e.g. nanowires, nanocages, nanocombs, nanodiscs, nanospings etc. A ZnO nanowire crystal is a hexagonal wurtzite structure. Zinc oxide crystal has oppositely charged planes (positively charged zinc ions and negatively charged oxygen ions). Such planes arrangement, causes a normal dipole moment—a spontaneous polarization along the c–axis [18,47]. The use of one–dimensional structures, such as zinc oxide nanowires, significantly increases the surface to volume ratio, which is very important in case of its photocatalytic and antibacterial properties [48]. There are many methods for zinc oxide nanowires synthesis, e.g. metal–organic chemical vapor deposition, electrochemical deposition, pulsed laser deposition and a hydrothermal growth method [20,21,48–50]. The conventional methods for ZnO nanowires synthesis are high–temperature processes [51]. Alternative, low temperature methods of ZnO nanowires production are methods of hydrothermal growth (HT) or chemical bath deposition (CBD). Among methods of zinc oxide nanowires hydrothermal growth, the sol–gel method strategy allows to bypass difficulties such as: high temperature or the use of vacuum [51]. Prior to the hydrothermal growth process, surface must be nucleated with zinc oxide nuclei. The nucleation process is frequently critical in the growth process, and it can decide on the success of modification. Nucleation can be conducted using e.g. spin coating or dip coating methods [21]. Zinc nitrate (source of Zn²⁺) and hexamethylenetetramine (HMTA) are the most commonly used precursor of hydrothermal growth. The ZnO nanowires growth occurs in temperatures ranging from 55 °C to 95 °C. In the literature, mechanism of ZnO nanowire growth is described in more detail [20,22,49]. On the other hand chemical bath deposition (CBD) is one of the simplest methods of zinc oxide deposition. It consists in a controlled chemical reaction (usually in an aqueous environment), through which a thin film of semiconductor is deposited on the substrate. Hydrophilic surfaces are preferred in case of hetero–nucleation, because the deposition takes place in aqueous solutions. Also adhesion of deposited semiconductor is much better on rough surfaces. In this method as a chelating agent ethanolamine in an ammoniacal solution was used [52,53]. Kokotov et al. [52,53] suggest using iron or manganese oxides or hydroxides as a nucleus for zinc oxide nanowires growth. ZnO nanowires growth can be controlled through change of ammonia concentration, as well as process temperature. Reducing the ammonia concentration may cause faster consumption of zinc ions, stemming from the rapid homogeneous precipitation of zinc oxide [52,53].

2. Materials and methods

2.1. Materials

Potassium permanganate, n–butanol, ammonia, methylen blue, hydrochloric acid, sodium hydroxide, glucose, disodium hydrogen phosphate heptahydrate, monobasic potassium phosphate, sodium chloride, ammonium chloride, magnesium sulfate, calcium chloride, dithizone were purchased from Chempur (Poland), Dissolution buffer 4.5 (acetate), sodium thiosulphate p. a., zinc sulfur and chloroform were obtained from Poch (Poland). Ethanolamine and ethanol were obtained from Avantor Performance Materials Poland S.A. (Poland). Zinc acetate and humic acid were purchased from Sigma–Aldrich. Agar and Lysogeny Broth were obtained from A&A Biotechnology (Poland). Bacteria strains E. coli PCM2560 and B. subtilis PCM2021 were obtained from Institute of Immunology and Experimental Therapy Polish Academy of Science (Poland). Crystal violet, safranin and iodine were purchased from Labo–mix (Poland).

2.2. Membrane used for modification

Microfiltration capillary polypropylene (PP) membranes ACUREL PP®V8/2HF (MEMBRANA GmbH) were used for further modification. The main properties of used membranes are presented in Table 1.
Table 1
Main properties of polypropylene membranes ACUREL PP®V8/2HF (MEMBRANA GmbH) [54].

<table>
<thead>
<tr>
<th>Properties</th>
<th>MEMBRANA GmbH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pore size</td>
<td>0.2 µm</td>
</tr>
<tr>
<td>Burst pressure</td>
<td>&gt;8 bar</td>
</tr>
<tr>
<td>Implosion pressure</td>
<td>&gt;4 bar</td>
</tr>
<tr>
<td>Outer diameter</td>
<td>2.6 mm</td>
</tr>
<tr>
<td>Inner diameter</td>
<td>1.8 mm</td>
</tr>
<tr>
<td>UFC</td>
<td>2.0 ml/bar cm²·min</td>
</tr>
<tr>
<td>Advancing contact angle</td>
<td>134.9°</td>
</tr>
</tbody>
</table>

2.3. Membrane plasma activation

Polypropylene membranes in some cases were treated by argon plasma, prior to nucleation and growth of zinc oxide nanowires. Plasma activation leads to introduction of new functional groups and the increase in membrane hydrophilicity and roughness, which are important factors in further modifications [55]. Plasma activation was carried out at the Institute of Electronic Materials Technology (ITME) in Warsaw. A plasma reactor by DIONEX Series 2000 Plasma Processing Reactor Center was used. Membranes were subjected to argon plasma for set amount of time in 25 W or 50 W, as a result of which, free radicals are formed on its surface. After activation, membrane modification was terminated in air.

2.4. Nucleation

During research both unmodified polypropylene membranes, as well as plasma–treated membranes were used for nucleation by synthesized manganese dioxide. Membranes were placed in 5 or 50 mM solution of potassium permanganate with addition of different amount of n-butanol for 25 min in 85 °C for nucleation. Loosely bound or unbound manganese dioxide nanoparticles were removed from the membrane surface, together with the unreacted potassium permanganate by RO water wash and ultrasound bath for 5 min. Such modification procedure is based on Kokotov et al. [52,53]. Kokotov et al. [52,53] stated that, during the creation of manganese dioxide by reduction method, manganese hydroxy-oxides (MnO(OH)) is most probably produced.

2.5. Zinc oxide nanowires growth by chemical bath deposition (CBD)

Nucleated membranes were subjected to the chemical bath deposition method of zinc oxide nanowires growth. Growth of ZnO nanowires was conducted in a solution containing 0.1 M zinc ions from zinc acetate, 0.5 M ammonia, and 1.7 M ethanolamine for set amount of time in 85 °C. Loosely connected ZnO nanowires were removed from the membrane surface in ultrasound bath. The method was based on Kokotov et al. [52,53].

2.6. Membrane characterization

Plasma treated, nucleated and membranes after ZnO nanowires growth were characterized by scanning electron microscope (SEM) and Fourier–transform infrared (FT-IR) spectrosopy. SEM analysis was carried out using Phenom G2 (Phenom World). The FT-IR analysis of the samples was obtained in the wavelength between 400 and 4000 cm⁻¹ using NICOLET 6700. Analysis was conducted according to the correlation tables [56]. In case of plasma treated membranes also measurement of wetting contact angle was performed. Contact angle was determined thanks to dynamic Wilhelmy method on K121 tensiometer (Kruss). XRD measurements were performed in classical focusing geometry Bragg-Brentano, using D5000 diffractometer (Bruker-AXS) equipped with a detector strip LynxEye (Bruker) in order to determine crystalline structure of obtained ZnO nanowires. In the measurements a copper X-ray tube in the optical system of a discrepancy 10 and Ni filter (1:20) were used. Goniometer radius R = 220 mm. The lamp Cu charged 40 mA current and voltage of 40 kV stability 0.01%/8 h. Amount of ZnO deposited on membrane surface was examined by dithizone method. Measured samples of membranes were placed in H₂SO₄ solution and sonicated for 10 min to dissolve all deposited ZnO. The dithizone method consists in the transfer of zinc ions from an aqueous solution into organic phase (chloroform), in which, along with dithizone, it forms a colored zinc-dithizone complex. Test samples were prepared by taking 2 ml of sample, adding sodium thiosulfate and the buffer maintaining pH at the level of 5. Next, a 0.002% dithizone solution in chloroform was added and shaken vigorously for 5 min. After 10 min, a color zinc-dithizone complex was collected and measured spectrophotometrically at wavelength of 620 nm. Zinc ion content was determined according to a standard curve prepared each time before test [57].

2.7. Determination of photocatalytic properties

In photocatalytic tests 2 mg/l methylene blue with different pH (3, 6) was used as model organic pollutant. Photocatalytic properties were confirmed by the methylene blue degradation. Modified membranes were placed in a laboratory module and then put in the installation where methylene blue decolorization occurred (Fig. 4). Modified membranes that were in direct contact with methylene blue were illuminated with four LEDs (emitting white light with a wide spectrum). Samples were collected every 20 min and concentration of methylene blue was measured spectrophotometrically at 663 nm wavelength. Also test for 2 mg/l humic acid removal in low pH (3) was performed. In this case membranes were illuminated with UV LEDs. Samples were also collected every 20 min. Concentration of humic acid was measured spectrophotometrically at 254 nm wavelength.

Table 2
Correlation table for FT-IR analysis of biological samples [58].

<table>
<thead>
<tr>
<th>Wave numbers (cm⁻¹)</th>
<th>Definition of the special assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3290</td>
<td>N–H and O–H stretching vibration: polysaccharides and proteins</td>
</tr>
<tr>
<td>2920</td>
<td>CH₃ symmetric stretch: mainly lipids, with minimal contribution from proteins, carbohydrates, and nucleic acids</td>
</tr>
<tr>
<td>2850</td>
<td>CH₃ symmetric stretch: mainly lipids, with minimal contribution from proteins, carbohydrates, and nucleic acids</td>
</tr>
<tr>
<td>1654</td>
<td>amide I (protein C=O stretching); α helices</td>
</tr>
<tr>
<td>1539</td>
<td>amide II (protein N–H bond, C–N stretch); α helices</td>
</tr>
<tr>
<td>1462</td>
<td>CH₃ bonding: lipids</td>
</tr>
<tr>
<td>1396</td>
<td>COO– symmetric stretch: amino acid side chains and fatty acids</td>
</tr>
<tr>
<td>1238</td>
<td>PO₃⁻ asymmetric stretch: mainly nucleic acid chains with little contribution from phospholipids</td>
</tr>
<tr>
<td>1084</td>
<td>PO₃⁻ symmetric stretch: nucleic acid and phospholipids</td>
</tr>
</tbody>
</table>
2.8. Determination of antibacterial properties

In case of all bacteriological experiments, overnight bacterial cultures were used. Plate antibacterial/bacteriostatic test was performed on model gram-negative and -positive bacteria strains (E. coli PCM2560 and B. subtilis PCM2021 respectively). A layer of a solid Lysogeny Broth (LB) containing 1% of agar was placed in Petri dishes with 100 μl of the appropriate bacteria culture. After semi gelation of medium sterilized membranes (polypropylene, polypropylene with ZnO nanowires, plasma treated polypropylene with ZnO nanowires) were placed in medium; so prepared plates were incubated for 24 h at 37 °C in presence of fluorescent light. The clear area (zone of inhibited growth) where no bacteria growth can be observed confirms bactericidal or bacteriostatic properties of tested material. In order to measure cell viability the cell density (OD550) measurement of 24–hour culture was performed. The LB and minimal (M9) broth were inoculated with bacteria strains in order to obtain initial OD550: 0.01 or 0.2. All samples were measured spectrophotometrically in sterile conditions at wavelength of 550 nm. OD550 was determined for cultures incubated for 24 h at 37 °C in presence of fluorescent light in 8 ml of sterile LB and M9 broth. In case of both bacteria strains and broth, amount of colony forming units (CFU) was obtained. First, Petri dishes with solid medium were performed (LB broth with addition of agar); once the medium solidified in sterile conditions 100 μl of bacteria cultures proper dilutions (strains incubated with all types of membranes in both types of broth for 24 h at 37 °C in presence of fluorescent light) was added and uniformly spread. After incubation Petri dishes were placed in incubator for 24 h at 37 °C. Then number of colony forming units (cfu/ml) was calculated. Cultures incubated with all kinds of membranes and in both types of medium (LB and M9) were stained according to gram staining procedure (application of crystal violet, application of iodine, ethanol wash, application of safranin). Then stained bacteria were observed under Eclipse E200 (NIKON) optical microscope. Also Growth inhibition curve was obtained. The set amount of a membrane was placed in 8 ml of sterile LB medium, and then the broth was inoculated with E. coli (initial OD550 = 0.2). Cultures with membranes were then placed for 24 h in an incubator at 37 °C (with fluorescent light). In sterile conditions, samples were taken, measured and returned every hour, and later in longer periods of time. In such way their OD550 was obtained. In case of E. coli incubated with modified membranes the FT–IR analysis of broth after bacteria growth was performed. In order to obtain FT–IR spectrum the organic matter was centrifuged so, as not to damage the bacteria. The authors attempted to demonstrate the differences in the composition of the medium after incubation with membranes. The baseline for FT–IR analysis was performed for the sterile LB broth. The analysis of FT–IR spectra was based on correlation tables (Table 2) [58].

Foster et al. [29] in their work show that a material (membrane) with photocatalytic properties may not only lead to cell damage or death, but also can cause it’s partial or complete mineralization.

Fig. 4. (a) scheme of stand for methylene blue or humic acid degradation via modified membranes (b) image of laboratory module.

3. Results and discussion

3.1. Modification and membranes characterization

Activation of polypropylene membranes was carried out in argon plasma. SEM images did not show noticeable differences in the visible morphology of membrane outer surface, no matter what plasma energy was used. Polypropylene membranes advancing contact angle in water is c.a. 135°, in case of polypropylene membrane after plasma treatment advancing contact angle was reduced even to c.a. 107°. Plasma surface activation is a two–step process. Firstly, the membrane is subjected to activation with an ionized gas, as a result of which, free radicals are formed on its surface. The second step of plasma surface activation consists in termination—exposure to e.g. air or other substances (gasses, liquids, vapors). After termination, new functional groups can be formed on the membrane surface [47]. Reduction of advanced contact angle (increased hydrophilicity) is caused by implantation of hydrophilic functional groups (Fig. 5) (hydroxyl or carboxyl) onto membrane surface [59]. Increase in membrane hydrophilicity should improve nucleation and modifications with ZnO nanowires, as they are carried out in aqueous solutions. FT–IR analysis confirmed some changes in membranes. Fig. 5 presents spectrum obtained for native polypropylene membrane and PP membrane treated in highest argon plasma energy. In case of plasma treated membranes, some additional small peaks can be observed (3500–3000 cm⁻¹ peak from –O–H bond; 1760–1690 cm⁻¹ peak from carbonyl bond –C=O). Presence of both hydroxyl and carbonyl group may indicate that some amount of carboxyl groups is present on membrane surface. Oh et al. [60], Oehr et al. [61] and D’Agostino et al. [62] also discuss the possibility of increasing the polymer hydrophilicity by plasma treatment.

In case of membrane modification with ZnO nanowires heteronucleation was used. Both, plasma treated and polypropylene membranes were nucleated by reduction of 5 mM or 50 mM potassium permanganate with addition of alcohol. Then nucleated PP membranes were subjected to growth of ZnO nanowires by chemical bath deposition method (CBD). In case of all membranes nucleated by reduction of 5 and 50 mM KMnO₄ even and uniform growth of zinc oxide nanowires was observed. Concentration of nucleating agent seems to be important. As in case of membrane nucleated by 5 mM KMnO₄ with addition of alcohol, observed zinc oxide nanowires are much shorter than those where nucleation was performed by 50 mM KMnO₄ with addition of alcohol. In case of membranes treated with plasma, some ZnO nanowires can be
observed even inside membrane surface pores. In Fig. 6 SEM images obtained for both untreated polypropylene membranes and membrane treated with highest plasma energy, as well as SEM images of membranes nucleated by 5 and 50 mM KMnO₄ with addition of n–butanol and after ZnO nanowires growth by CBD method are presented.

FT–IR analysis was performed for membranes modified with ZnO nanowires and for clean polypropylene membrane. Fig. 7 presents the spectrum obtained for a PP membrane and PP/ZnO nanowires membrane. In case of PP/ZnO spectrum in the wavenumber range from 400 to 700 cm⁻¹, there is a clear peak, which is not present for the spectrum for polypropylene membrane, which might prove presence of zinc oxide on the membrane surface.

Comparison of XRD spectrum for PP and PP/plasma/ZnO membranes (Fig. 8) display very similar elements of the polypropylene structure with clearly visible additional diffraction patterns of wurtzite [63,64] (magenta curve): <100> – 32° ; <002> – 34° ; <101> – 36° ; <102> – 47.5° ; <110> – 56° ; <103> – 63° and <112> – 67°. Comparison of both modified membranes does not show significant discrepancies in peaks distribution, however differences in peak heights are noticeable. Peaks from sample with plasma pre-treatment are higher, which may indicate larger amount of ZnO nanowires on membrane surface. Those results corresponds to the determination of Zn ions concentration by dithizone method. In case of membrane modified with zinc oxide nanowires without plasma pretreatment, the amount of Zn is around 0.037 mg/cm² and 0.053 mg/cm² for membranes with plasma pretreatment and ZnO nanowires. The width of the first phase of reflections (Scherrer formula) suggest the shape
3.2. Determination of photocatalytic properties

Degradation of 2 mg/l methylene blue (MB) in different pH, was used to prove photocatalytic properties of membranes modified with ZnO nanowires (after plasma treatment). Fig. 11 a shows a relationship between C/Co and time of methylene blue decolorization in contact with polypropylene membrane and membrane modified with zinc oxide at pH 3 and 6. In case of polypropylene membrane mainly photolysis and sorption of methylene blue can be observed. The main loss of methylene blue concentration occurs during first 20 min. In case of membranes modified with ZnO nanowires at low pH the biggest loss of methylene blue concentration can be observed and degradation rate is 0.0214 min⁻¹, but at neutral pH the methylene blue degradation rate is 0.0143 min⁻¹. Baruah et al [30] stated that zinc oxide nanowires, can degrade methylene blue (10 μM) with degradation rate varying from

Fig. 10. Photoluminescence of membrane modified by ZnO nanowires.

Fig. 11. Graph representing the dependence of: (a) methylene blue ln(C/Co) from time; (b) methylene blue decolorization with time.
0.005993 to 0.012792 min$^{-1}$. Degradation rate strongly depend on method of ZnO nanowires formation [30].

Fig. 11b shows the degree of methylene blue decolorization (\((I_{0} - I) / I_{0}\) × 100) with time, in contact with polypropylene membrane and membrane modified with zinc oxide at pH 3 and 6. For polypropylene membrane the methylene blue solution lost ~20% of its color. As for PP membrane modified with ZnO nanowires at pH = 6, within the first 40 min of the process methylene blue lost ~60% of the color, and then during the next 60 min—a little over 10%. However, in case of the same membranes in low pH methylene blue lost ca. 80% of color during first 60 min and later only about 10%. Test showed that lower pH value of the degradation environment gives better results. Value of pH significantly influences the physicochemical properties of semiconductors, such as zinc oxide, including charge of particle and position of the conduction and valence bands. Schmelling et al. [65] depicted that a change in pH causes a shift in Fermi level of the semiconductor so that the photocatalyst becomes a better oxidant with decreasing pH value. The obtained results present a similar tendency, as those discussed by Baruah et al. [30], Balta et al. [34] and Wang et al. [66] in their works. In case of humic acid (low pH) degradation in presence of UV LEDs by membranes modified by ZnO nanowires, loss of about 50% of humic acid concentration was observed. It can be concluded that membranes modified with zinc oxide nanowires exhibit photocatalytic properties. Therefore it can be assumed that membranes modified in such way will also exhibit antibacterial properties because of reactive oxygen species production.

3.3. Determination of antibacterial properties

For testing antibacterial properties model gram(+) and gram(−) bacteria (B. subtilis and E. coli respectively) were used as well as polypropylene membranes (marked as PP), polypropylene membranes nucleated by reduction of 50 mM by alcohol and after ZnO nanowires growth by CBD method (marked as PP/ZnO) and plasma treated (highest plasma energy) polypropylene membranes nucleated by reduction of 50 mM by alcohol and after ZnO nanowires growth by CBD method (marked as PP/plasma/ZnO).

The bacteriostatic or bactericidal properties of membranes were proved, if near tested material the growth inhibition zone were present (transparent zone). Fig. 12 presents plates incubated with polypropylene, polypropylene with and without plasma and ZnO nanowires. Both plates were incubated with (a) B. subtilis and (b) E. coli, and then incubated for 24 h in 37 °C with presence of fluorescent light. As it is presented in Fig. 12 in case of both bacteria strains no zone of inhibited growth is visible for polypropylene membrane. Clear zone of inhibit growth can be observed for membrane with ZnO nanowires for B. subtilis, but almost no zone of inhibited growth was present in case of E. coli. Very clear zone of inhibit growth can be observed for membrane after plasma treatment with ZnO nanowires for B. subtilis, but in case of E. coli zone of inhibited growth is very slim. In case of plasma–activated membrane with ZnO, the zone of inhibited growth is much bigger than in case of polypropylene membrane with ZnO. It can be assumed that modified membranes exhibit good antibacterial/bacteriostatic properties against B. subtilis in solid medium.

Antibacterial properties were also determined in liquid medium both for M9 and LB broth. Fig. 13 presents bacteria cell density \((\text{OD}_{550})\) after 24 h of incubation in 37 °C in relation to the initial concentration of E. coli in the LB broth. Three different initial concentrations were used \((\text{OD}_{550} = 0.01; 0.1; 0.2)\). As shown in the graph, regardless of used initial concentration, the presence of ZnO on the membrane surface reduction of bacteria cell density after 24 h is bigger, compared to a polypropylene membrane. In case of this experiment, a far better results were obtained for more severe conditions (higher initial bacteria cell density) for membranes that were first treated with plasma and then with zinc oxide nanowires.

Growth inhibition curve for E. coli in LB broth was obtained. Fig. 14 shows a graph of the growth inhibition curve for all kinds of investigated membranes. In case of all membranes phase of logarithmic growth can be observed. For PP membrane exponential growth of microorganisms is the fastest. For a membrane modified with ZnO, the rate with which the microorganisms grow decreased,
but the smallest bacteria growth rate was observed in case of plasma–treated membranes with ZnO. After first 5 h of E. coli propagation, the OD550 for a PP membrane is greater by over 0.4 than the OD550 for both membranes with ZnO membranes. The OD550 test does not distinguish dead form alive cells, that is why only an approximate determination of microorganism concentration is possible. To observe a drop in the OD550, the cell must be lysed.

According to the literature [29] the bacterial cell content can be mineralized by photocatalytic properties of semiconductor. The FT–IR analysis of the medium (LB) growth was conducted. After 24 h incubation of all types of membranes with E. coli, the bacteria were centrifuged, and the remaining medium subjected to FT–IR analysis. Fig. 15 presents the FT–IR spectrum for an unmodified PP membrane, a PP/ZnO and PP/plasma/ZnO membrane. It is clearly visible that the spectra for membranes containing ZnO are similar. The FT–IR analysis was conducted according to correlation tables presented by Tang et al. [58], and Liu et al. [67]. In case of membranes after ZnO growth, peak in the region of 2920 cm⁻¹ and 2850 cm⁻¹ disappears (CH₂ bond (possibly from lipids or to a small extent from proteins, carbohydrates or nucleic acid)). In the region of 1651 cm⁻¹, for ZnO membrane is more visible than in case of PP membrane (C=O bond from the primary amides (proteins)), but at the same time the peak in the region of 1539 cm⁻¹ disappears (secondary amides, i.e. the NH, CH bond in proteins). Then, in the vicinity of 1462 cm⁻¹ for membranes after ZnO growth, the peak is reduced (CH₂, derived from lipids). In the vicinity of 1396 cm⁻¹ for membranes with ZnO, the peak disappears (COO⁻, derived from amino acids (side chains), or fatty acids). For the wavelength of 1238 cm⁻¹, the peak disappears, and for 1084 cm⁻¹, the peak is also reduced for membranes modified by ZnO (PO₃⁻, derived from nucleic acids and phospholipids). The exact products of photocatalytic degradation of microorganisms are very difficult to define [41,63]. Based on obtained data (decrease or disappearance of peak), we can conclude decay of lipids, carbohydrates and amino acids as well as decomposition of secondary to primary amides, which indicates cell lysis and partial mineralization of cell organic compounds by zinc oxide nanowires (Fig. 16).

For B. subtilis incubated with all kinds of membranes in LB and M9 broths, colony forming units (CFU) were counted. Fig. 17 shows the amount of CFU ml⁻¹ for each sample. In case of PP/ZnO membrane, decrease of CFU is observed for both broths. For LB around two orders of magnitude and for M9–ca. three orders of magnitude. For PP/plasma/ZnO membrane, decrease of CFU is also observed for both broths. For LB around five orders of magnitude and for M9–no bacteria colony was observed, which may indicate that broth was completely sterilized.

Microscopic pictures of LB broth after B. subtilis incubation are presented in Fig. 18a–c with PP, PP/ZnO and PP/plasma/ZnO respectively. All bacteria samples were stained with Gram method. The biggest amount of bacteria is visible in case of PP membranes for LB broth. In case of PP/ZnO membranes, the amount of bacteria is much smaller and the smallest amount is for PP/plasma/ZnO. Also microscopic pictures of M9 broth after B. subtilis incubation are presented in Fig. 18d–f with PP, PP/ZnO and PP/plasma/ZnO. The biggest amount of bacteria is visible in case of PP membranes for LB broth. In case of PP/ZnO membranes only few bacteria are visible and in case of PP/plasma/ZnO almost no bacteria can be observed.

Obtained data confirmed the information on antibacterial properties of zinc oxide nanowires available in the literature [30,32,34].
also immobilization of zinc oxide nanowires on the membrane surface did not decrease ZnO nanowires antibacterial properties. It can be assumed that ZnO nanowires exhibit antibacterial properties against both gram(+) and gram(−) bacteria as well as spores which was also confirmed by Azam et al. [33]. According to literature, *B. subtilis* seems to be more sensitive to ZnO nanowires, which was also confirmed by Adams et al. [30]. The presented results enable the assumption that modified membranes will not let the biofilm to grow at the time of installation downtime, and therefore reduce the necessity to wash the membranes (both chemically or physically).

**4. Conclusions**

It is possible to uniformly cover polypropylene capillary membranes by ZnO nanowires by chemical bath deposition method after heteronucleation. Presented results allow the conclusion that modified membranes exhibit both photocatalytic and antibacterial properties against gram−positive and −negative bacteria. The use of plasma, as the initial step of modification, improved the deposition of zinc oxide nanowires on the membrane surface thus increasing its antibacterial properties. Such membranes may have the ability to reduce problem of fouling both organic as well as microbiological. Also presence of ZnO nanowires on membrane surface should slow down or even prevent the formation of biofilm on membrane surface during downtime of installation.

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**References**


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