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Biocide modification of ultrafiltration membranes using nanofiber structures

J. Dolina[a,*], T. Jiříček[a], T. Lederer[a,b]

[a]*Centre for Nanomaterials, Advanced Technologies and Innovation, Technical University of Liberec, Studentská 1402/2, 461 17 Liberec, Czech Republic, Tel. +420 485353874; email: jan.dolina@tul.cz (J. Dolina), Tel. +420 485353650; email: tomas.jiricek@tul.cz (T. Jiříček), Tel. +420 603242832; email: tomas.lederer@tul.cz (T. Lederer)*
[b]*Aquatest a.s., Geologická 4, 152 00 Praha, Czech Republic, email: tomas.lederer@tul.cz*

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ABSTRACT

Membrane biofouling has a negative effect on membrane performance. Commercial ultrafiltration (UF) membranes were therefore modified using polymeric nanofibers in order to gain additional water treatment functionality. In this case, membrane surface was modified using biocidal electrospun polyurethane nanofibers. It has been found that the inorganic silver in form of reduced silver nitrate is a feasible modification of nanofiber structure, and electrospinning conditions were optimized to maximize silver particle content. Two possible methods were verified for preparing composite UF membranes with antibacterial properties using: (a) direct nanofiber deposition and (b) thermal pressure lamination. Hydrodynamic properties of the developed membranes were tested on an AlfaLaval M10 unit. It has been found that the added functionality comes at the expense of lower permeability. Biocide properties over both short- and long-term operation were confirmed through cultivation techniques and respirometry with *Escherichia coli* while concurrently monitoring how the added silver leached from the nanofiber layer. The results indicate that UF membranes modification using silver-treated nanofibers is a promising technique worth future study and evaluation for advanced water treatment.

Keywords: Composite membranes; Biofouling; Ultrafiltration; Nanofibers; Silver; Biocide properties; Water treatment

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1. Introduction

Recent scientific research on ultrafiltration (UF) membranes has concentrated on modifications to improve filtration characteristics at the nano-level. The pores in an ideal membrane should remain unblocked throughout the filtration process; pore-blocking, however, frequently occurs due to passage of both inorganic and organic compounds, including metabolism by-products of micro-organisms [1]. The prevention of microbial growth on a membrane's surface, therefore, is essential in order to avoid frequent chemical and mechanical membrane cleaning.

In this study, we focus on nanofibers with incorporated silver nanoparticles [2] and integration of such nanofiber layers into membrane topcoats in order to

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*Corresponding author.

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increase antibacterial properties. This type of modified membrane is proposed for filtration of organically contaminated water and for possible use in membrane bioreactors.

2. Materials and methods

2.1. Materials

2.1.1. Polymeric nanofibers

Nanofibers were prepared by electrospinning [3], whereby fibers are formed from a thin layer of polymer solution through the effects of an electrostatic field (Fig. 1). The capability of a polymer to form fibers is affected by a number of process and system parameters, such as the intensity of electric field, viscosity, molar mass, surface tension, and polymer concentration. Polyurethane (PU) nanofiber layer were used in these experiments: PU is the most abrasion resistant elastomeric polymer, displaying mechanical stability, good chemical resistance, and long-term heat resistance. Its hydrolytic stability surpasses that of polymeric amides. Further, PU nanofibers have known hydro-mechanical properties and a proven ability to be electrospun [4].

The PU used for nanofiber production was based on a 30% standard solution (Larithane®) in dimethylformamide (DMF; Penta; ≥99.5%) provided by Novotex.

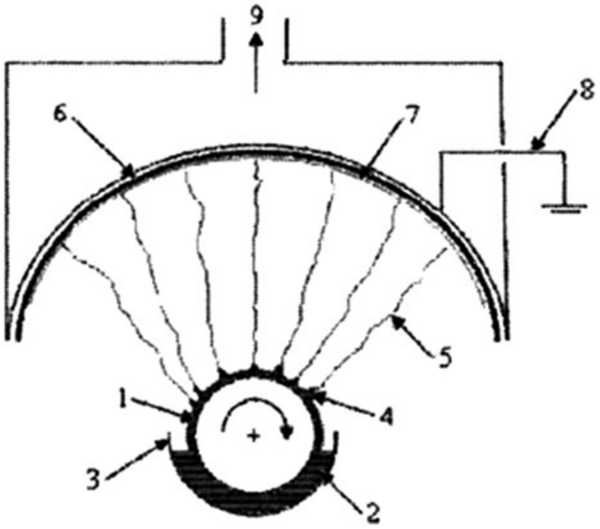


Fig. 1. Free surface electrospinning device illustration [9]. Description: (1) metal roller, (2) polymer solution, (3) solution reservoir, (4) Taylor cone, (5) fiber casting direction, (6) support material, (7) nanofiber sheet, (8) ground electrode, and (9) air conditioning.

2.1.2. Silver addition and reduction

The silver precursor in form of silver nitrate ($AgNO_3$; Penta; ≥99.8%) was tested. Compared to ionic silver, zero-valent silver shows lower cytotoxicity, increased antibacterial activity, and lower coagulation in environments with high ionic strength [5]. The reduction of silver was made *in situ* by using dimethylformamide [6] as a solvent and by application of long wave UVA [7] light (315–400 nm) by using Philips TUV 25 W lamp after fiber formation.

2.2. Methods

2.2.1. Preparation of nanofiber solutions

The PU standard solution, silver solution and solvent or reducing agent were mixed together; following which a solvent was added in order to obtain a polymer concentration (typically 14–18%) and viscosity suitable for nanofiber electrospinning. This solution was then stirred overnight. Immediately before electrospinning, the samples were placed into an ultrasound bath and heated to 65˚C for approximately 15 min to increase polymer chain mobility.

2.2.2. Nanofiber casting method

Large-scale samples were later produced by continuous electrospinning [8]. The nanofiber solution was placed in a reservoir with a positively charged metal round roller (Fig. 1). For a high voltage source, the Spelmann SL 600 W device was used.

PU nanofibers with reduced silver nitrate were obtained under the following process conditions: voltage 68–72 kV; electrode distance 0.16 m; RH 18–22%; roller (winding) speed 0.05 m/min; and temperature 26.0–28.0˚C. The solution was cast onto polypropylene spunbond (Pegas; non-woven).

2.2.3. Morphological analysis and particle size distribution

Morphological analysis was undertaken using the FEI Phenom™ scanning electron microscope (SEM) in the Department of Non-woven Textiles of the Technical University in Liberec (TUL), and a JEOL JSM-6340F microscope, with the co-operation of the Flemish Institute for Technological Research (VITO). The nanofibers were scanned either uncoated, or coated with a 2.5 nm layer of platinum (Pt)/palladium (Pd) (80/20), and images obtained at an acceleration voltage of 20 and 5 kV, respectively. The particle size distribution was evaluated by using NIS-Elements AR 3.10 (Nikon) software.

2.2.4. Preparation of composite membranes

A number of nanofiber layer thermal pressure fixation experiments were performed on flat sheet Microdyn-Nadir UP150 PES UF membranes [10] (150 kDa; polypropylene (PP)/polyethylene teraphthalate substrate). Lamination tests were undertaken using an OSHIMA OP-450GS Mini Press (temperature 0–230°C; maximum pressure 0.14 MPa), with Teflon™ surface coating on the molding strip. Continuously electrospun nanofiber layers were thermally detached from the support layer and laminated onto the membrane's surface in order to complete the membrane composite.

2.2.5. Nanofiber deposition directly onto the membrane's surface

In order to avoid membrane degradation caused by lamination, we also examined direct fiber deposition onto the membrane's surface by electrospinning. Nanofibers prepared under the correct electrospinning conditions [11] are able to show excellent adhesion to the test surface. Direct deposition was undertaken by attaching a membrane to the PP non-woven substrate and casting nanofibers by continuous electrospinning.

2.2.6. Membrane testing

2.2.6.1. Membrane permeability. Permeability measurements were undertaken using a 50 ml Millipore/Amicon 8050 dead-end cell driven by nitrogen pressure and an Alfa Laval M10 cross-flow unit, operated under constant volume operational mode and using DEMI water at room temperature as the filtration liquid.

2.2.6.2. Leaching of silver from the nanofiber layer. Two modes of silver stability were tested, (a) silver concentration in permeate measured through filtration of 50 ml of DEMI water through dead-end and cross-flow units, and (b) based on leaching from dry samples measured by agitating the modified membrane in DEMI water. An exact volume was taken at regular intervals for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis, i.e. 0, 5, 15, 30, 60, and 120 min for short tests; and 0, 1 h, 1 d, and 4 d for medium term tests. Integrated sampling involved one volume per each membrane and samples were taken from the continuously concentrating liquid. For the derivation method, the same membrane was placed in a new volume of DEMI water following each sample and the respective silver concentrations added together. For both methods, the resulting total silver content should be identical.

Nitric acid was added to each sample volume to obtain a 1% acidic solution and the sample was then analyzed on an ICP Optima 2100 DV spectrometer with Argon as the inert phase and calibration set by the 0.1 tree point and 10 mg/L standard curve.

To determine the total amount of silver, the nanofiber structure was separated from the carrier and, following mineralization, the samples were converted to a 50 ml volume and the absolute concentration calculated.

2.2.6.3. Antimicrobial testing. The antibacterial properties of the composite membranes were tested by (a) filtering *Escherichia* coli (reference number according to ČSNI: CCM 3954) bacterial solution through the membrane in a dead-end and cross-flow arrangement, then cultivating the bacteria on the membrane's surface and observing growth; and (b) using an online respiration activity monitoring system. Oxygen transfer rate was found to be the most suitable parameter for quantifying the physiological state of the aerobic micro-organism culture as most metabolic activities depended on oxygen consumption [12]. These measurements were undertaken in co-operation with the NAMETECH project partner, RWTH Aachen University.

For midterm leached membranes we use this biocide test: *E. coli* bacteria (CCM 3954) were inoculated onto growing plate count agar substrate. After significant growth, bacterial forming units were dispersed into saline and the absorbance measured at 600 nm (OD 600) on a Hach-Lange DR2800 laboratory analysis spectrophotometer. This wavelength corresponds to the inner-phase of *E. coli* growth. For cell calculation, we use linear relation [13] that saturated *E. coli* culture has about 1×10^9 cells/ml.

We diluted the initial solution to get approx. 1×10^5 cells/ml (A), and 1×10^6 cells/ml (B). A 10 ml sample of A was then added to two 2 × 2 cm silver-coated membranes, and 10 ml of B into a third sample. The membranes were previously leached for 4 d, following which they were intensively washed with demineralized water to ensure that no previously leached silver remained attached to the membrane's surface. We used two blank samples of saline for comparison.

The *E. coli* samples were incubated for 24 h at 37°C, while the samples were stirred by hand at 0, 12, and 24 h. Subsequently, 1 ml of 0, −1, −2, −3, and −4 log diluted samples was placed on agar plates filled with plate count agar medium without dextrose. The samples were then kept under aerobic conditions for 48 h at 37°C for cultivation.

3. Results and discussion

3.1. PU nanofiber layer

The addition of silver particles to the spinning solution negatively affected both homogeneity and fiber structure, resulting in a wider fiber diameter compared to pure PU nanofibers. The surface charge of silver particles had a major impact by limiting the mobility of the charged polymer group when the electric field was applied. Dilute silver solutions ensured easier electrospinning as the required conductivity could be maintained longer; however, it also resulted in lower concentrations and uneven particle distribution. Higher silver concentrations (depending on the solvent system used) tended to suppress electrospinning efficiency. The optimal silver concentration lay between these extremes.

The concentration of silver in PU nanofibers was relatively high (22% of polymer weight (wt_p)), with the specific concentration of silver reaching 800–1,200 mg/m^2 for nanofiber sheets prepared using continuous electrospinning and cast onto PP supports. At a winding speed 0.05 cm/min, the weight of the nanofiber layer following separation from the support was 6.5–7.2 g/m^2.

3.2. Morphological analysis and particle size distribution

The fiber diameter of PU layer was determined by image analysis to 240 ± 64 nm. Mean particle size for silver nanoparticles (Fig. 2) was determined to 80 ± 21 nm. Silver presence was confirmed by using X-ray diffraction particle spectra (Department of Chemistry, TUL).

3.3. Preparation of composite membranes

3.3.1. Thermal pressure lamination

Initial experiments on flat sheet membranes indicated that adhesion between nanofibers and the membrane was achievable. Increased adhesion through thermal pressure lamination, however, led to nanofiber and/or membrane degradation. In the case of PU nanofibes, structural loss was observed in the nanofiber layer at temperatures above 120°C.

The nanofiber layer was separated from the PP support layer shortly before the lamination step. Best lamination results in terms of adhesion and morphological changes were obtained in the temperature range from 95 to 100°C and at a pressure of 2.0 kPa. These modified composite membranes were tested further for permeability and antimicrobial properties.

3.3.2. Direct deposition of nanofibers on the membrane surface

SEM imaging confirmed that directly cast nanofiber layers were uniformly deposited (Fig. 3). The limiting factor during this procedure, however, is likely to be residual solvent content. While permeability declined using this procedure; the process resulted in a lower drop in permeability than when using thermal pressure lamination.

3.4. Reduction methods for silver nanoparticles

The reduction method of silver in dimethylformamide was proven as efficient for silver solvent reduction. This step is recommended before the polymer addition to solvent-reduction system. The

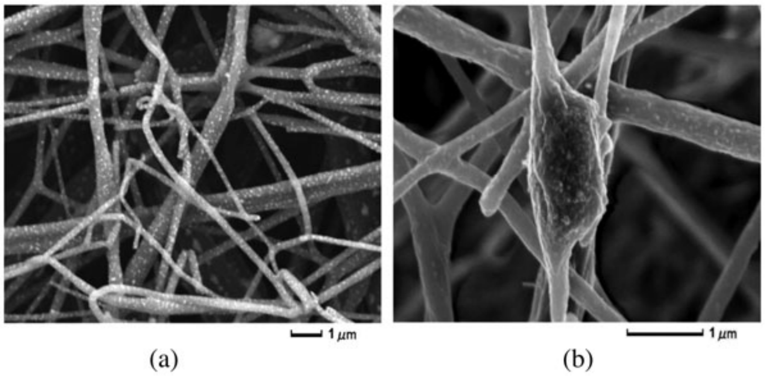
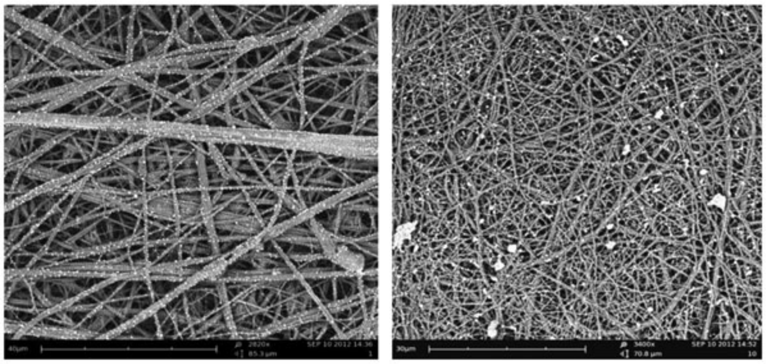


Fig. 2. PU nanofibers with silver nanoparticles. Images obtained by JEOL-JSM microscope; AV 5 kV; mag. (a) 10,000× and (b) 5,000×.



(a) (b)

Fig. 3. Scanning electron microscope images of dry samples after (a) 1 min, and (b) after 10 min in DEMI water; AV 20 kV; mag. (a) 2,820× and (b) 3,400×.

UVA ex-post reduction proved the most effective method in terms of lowest chemical influence on nanofiber morphology and color change efficiency.

3.5. Membrane testing

3.5.1. Membrane permeability

Both testing protocols confirmed that increased lamination temperature decreased permeability for both empty membranes and composite nanofiber membranes, with the latter more significant.

As both membrane and nanofibers suffered at high temperatures, two future concepts were assessed: (a) use of different polymers with good adhesion to PES membranes at lower temperatures, and/or (b) elimination of the lamination process completely.

PU nanofibers require lower temperatures for good adhesion (than in case of e.g. PES nanofibers), provide greater permeability. Permeability values were still lower than those of empty membranes, however, so a composite membrane without lamination was developed. Fig. 4 shows development of composite membrane permeability achieved without thermal pressure lamination. Typically, empty membranes had twice the permeability of laminated membranes. Casting PU nanofibers directly onto the membrane's surface, rather than through lamination improved permeability by about 50%.

As shown in Fig. 4, nanofiber layers added by thermal pressure lamination showed increased resistance compared with free deposition. Due to the very high porosity of nanofiber layers with a fiber diameter in the 200 nm range, resistance is expected to be orders of magnitude lower than that for 150 kDa UF

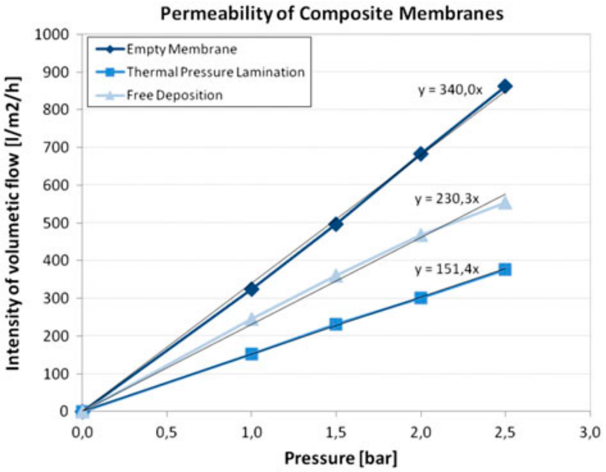
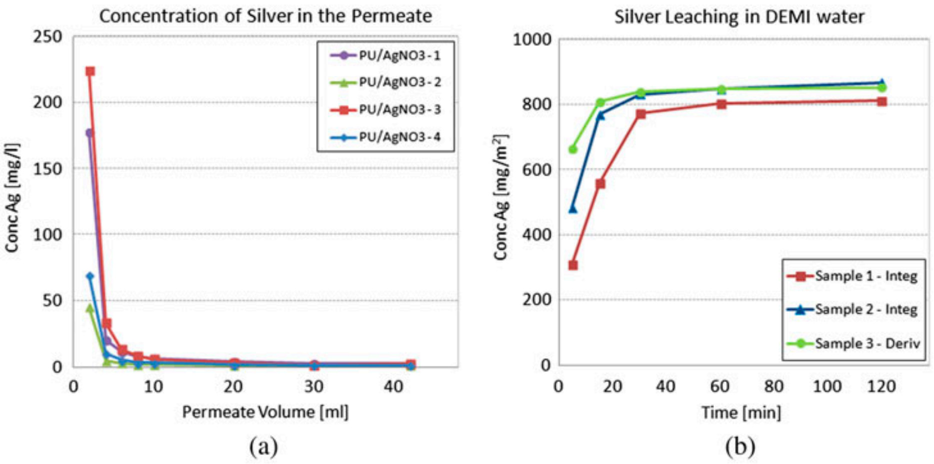


Fig. 4. Improved permeability achieved by direct deposition on a membrane's surface.

Table 1
E. coli bacteria count after 48 h cultivation for 0 to −4 log dilution. Samples 1–3 are the silver leached membranes. The initial *E. coli* cell concentration of sample 3 was 10× higher than 1 and 2. Samples 4 and 5 are blank samples.

	Sample				
Log dilution	1	2	3	4	5
0	2	2	2	364	247
−1	1	0	0	38	26
−2	0	0	0	4	2
−3	0	0	0	0	0
−4	0	0	0	0	0



(a) (b)

Fig. 5. (a) Leaching characteristics of silver at different initial concentrations and (b) silver leaching in DEMI water.

membranes. The increase in resistance can be explained by residual solvent flow, which could block some of the membrane pores and could transform some nanofibers into less porous interconnected structures.

Composite membrane permeability will never be as high as that of empty membranes; however, this is the price paid for the added antibacterial functionality.

3.5.2. Leaching of silver from the nanofiber layer

Our experiments indicate that silver is passed through a 150 kDa MWCO membrane, even though this is denser than the reported particle sizes listed in Table 1. This is due to the silver dissolving and silver ions passing through the membrane, not the particles themselves.

Excessive leaching of silver antibacterial particles from the nanofiber layer into wastewater is potentially a serious environmental problem, and a recognized drawback of present methodology. Further, excessive silver leaching reduces the long-term biocide properties of the nanofiber layer. For these reasons, we focused on the leaching characteristics of the nanofiber layer with the best antibacterial properties, i.e. PU nanofibers with silver nitrate on Nadir UP150. A very similar leaching pattern was observed, independent of the initial silver content (typically 1,200 mg/m^2) (Fig. 5(a)). Most of the silver was washed out in the first permeating milliliters, and was directly proportional to the initial amount of silver in the nanofiber layer. There was no difference in leaching between laminated and free deposition membranes. When left mixing in DEMI water, the concentration of silver stopped increasing after 1 h and remained constant at the expected amount, i.e. 800 mg/m^2, which corresponds to app. 66% of the amount of silver added during electrospinning (Fig. 5(b)).

The same experiment, extended to four days, showed that silver leaching stopped after approx. 1 h and from then on the concentration of silver remained constant. There was no significant difference between the integration and derivation methods, meaning that leaching did not stop due to concentration equilibrium on the nanofiber's surface and in water, but that the remaining silver was actually fixed on the nanofiber's surface, providing positive antibacterial activity (see biocide tests for 4 d leaching).

SEM scans indicated excess silver in the dry samples; with small silver particles visible on the fiber surfaces after 1 min (Fig. 5(a)), and aggregation of silver after 10 min (Fig. 5(b)).

3.5.3. Antibacterial properties (CFU)

In order to verify antibacterial stability over time, the leaching tests were carried out using 20× higher permeate volume and approx. twice the bacterial content (300 CFU to 336 cm^2 of membrane in Alfa Laval M10, as opposed to 5 CFU per 13.4 cm^2 using a dead-end Amicon cell). No bacterial growth was observed on the membrane using this arrangement.

The midterm antibacterial test of the count formation units per milliliter as shown in Table 1 proved the biocide effect of modified membranes after 4 d leaching.

3.5.4. Antibacterial properties—respiration activity

Three different silver-coated membranes (Nadir UP150) were tested against a blank sample (samples I and II used the same lamination conditions and were

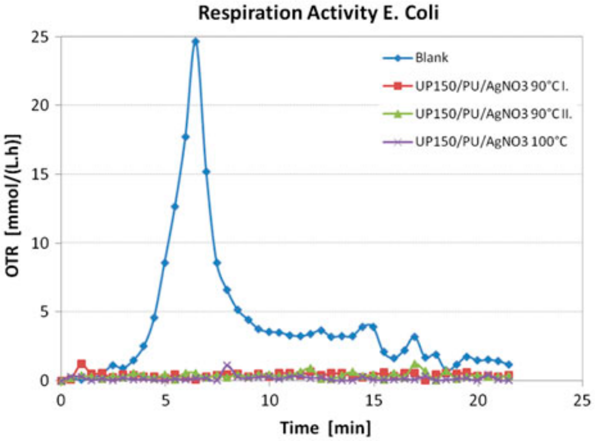


Fig. 6. Oxygen transfer rate of *E. coli* using three different silver-coated membranes.

used for reproducibility), with very strong antibacterial properties illustrated for all three membranes (Fig. 6).

4. Conclusions

It is possible to combine inorganic silver with nanofiber structures resulting in antimicrobial nanofibers for water treatment application.

There are two possible methods for preparing composite UF membranes with antibacterial properties, (a) direct nanofiber deposition, and (b) thermal pressure lamination. Added functionality, however, comes at the cost of lower permeability, which can be improved by optimizing the deposition process.

Antimicrobial properties over both short- and mid-term operation were confirmed using two different methods (CFU and respiratory), while concurrently monitoring how added silver leached from the nanofiber layer.

Future research will be based on; (a) optimizing silver content in nanofibers, (b) optimizing nanofiber diameter in order to minimize initial leaching, and (c) testing membranes under actual UF conditions and using actual wastewater.

Acknowledgments

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References

[1] L. Dvořák, M. Gómez, M. Dvořáková, I. Růžičková, J. Wanner, The impact of different operating conditions on membrane fouling and EPS production, Bioresour. Technol. 13 (2011) 6870–6875.

[2] J.R. Morones, J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramírez, M.J. Yacaman, The bacterial effect of silver nanoparticles, Nanotechnology, 16 (2005) 2346–2353.

[3] Z.M. Huang, Y.Z. Zhang, M. Kotaki, S. Ramakrishna, A review on polymer nanofibers by electrospinning and their applications in nanocomposites, Compos. Sci. Technol. 63 (2003) 2223–2253.

[4] R.B. Seymour, G.B. Kauffman, Polyurethanes: A class of modern versatile materials, J. Chem. Educ. 69 (1992) 909–914.

[5] A. Panacek, L. Kvítek, R. Prucek, M. Kolar, R. Vecerova, N. Pizúrova, V.K. Sharma, T. Nevecna, R. Zboril, Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity, J. Phys. Chem. Part B 24 (2006) 16248–16253.

[6] I.P. Santos, L.M. Marzán, Reduction of silver nanoparticles in DMF, formation of monolayers and stable colloids, Pure Appl. Chem. 72 (2000) 83–90.

[7] R.P. Chadal, S. Mahendia, A.K. Tomar, Effect of ultraviolet irradiation on the optical and structural characterics of *in situ* prepared PVP-Ag nanocomposites, Dig. J. Nanomater. Biostruct. 6 (2011) 299–306.

[8] O. Jirsák, F. Sanetrník, D. Lukáš, V. Kotek, L. Martinová, J. Chaloupek, A method of nanofiber production from polymer solution using electrostatic spinning and a device for carrying out the method, CZ Patent 294274 (B6), WO2005024101, 2009.

[9] D. Lubasová, L. Martinová, Electrospun Chitosan based Nanofibers, Technical University of Liberec RJTA 12 (2008) 72–78.

[10] MICRODYN-NADIR GmbH, Membrane Bioreactors: Microdyn-Nadir Product Solutions, D-65203 Wiesbaden, Germany, 2009. Available from: http://www.microdyn-nadir.com.

[11] Z. Tang, Ch. Qiu, J.R. McCutcheon, K. Yoon, H. Ma, D. Fang, E. Lee, C. Kopp, B.S. Hsiao, B. Chu, Design and fabrication of electrospun PES nanofibrous scaffold, J. Polym. Sci. Part B 47 (2009) 2288–2300.

[12] T. Anderlei, W. Zan, M. Papaspyrou, J. Buchs, Online respiration activity measurement (OTR, CTR, RQ) in shake flasks, Biochem. Eng. J. 17 (2004) 187–194.

[13] R. Phillips, J. Kondev, J. Theriot, Physical Biology of the Cell, Garland Science, London, 2008, p. 40.