BIOCIDAL EFFICIENCY ON CONSORTIUM ALGAE AND BACTERIA OF NANOFIBER TEXTILES DOPED BY BIOCIDE SUBSTANCE

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Abstract

That article is focused on the biocidal effect of nanofiber textiles based PVA enriched by agents with potential (copper, diamond and biocide) to limit the growth of consortium consists of algae and bacteria. It was proved that the basic PVA (solution and nanofiber textiles) has not any effect against the growth. The biocidal addition causes the inhibition the growth of consortium bacteria and algae.

Keywords: Algae, nanofiber textile, PVA, biocide

1. INTRODUCTION

The first visible organisms on building constructions are algae [1]. Algae have been most studied to connection with the building structures because they occur on coatings on a building stone or external envelope of building and create biofilms. Algae on building stone cause degradation by their metabolites and water retention, which is especially problematic in freezing cycles. Algae are commonly found in consortium with algae, which also alone degrade building materials. Classical protective treatments are carried out by conventional organic and inorganic substances which have efficiency depence in the time and on environmental conditions.

Algaecide protection of building materials haven't still used regularly or systematic. The protection treatments are more focused on all microorganisms in general. The used biocidal compounds are commonly chosen from next groups: metal, complex compounds, organic compounds or quaternary ammonium salt.

There are several studies focused on possibility to use nanotechnology for that purpose. Nanotechnology is one of the most cross-disciplinary dynamic developing field of science. The great interest is focused on the study of the production and application of nanofibers in last 15 years [2-3]. Produced nanofibers can reach smaller diameters, thereby is increased their high specific surface area [4].

2. APPLIED MATERIAL AND EXPERIMENTAL METHODS

Model organism

The model organisms were used a consortium which is occurred in the Czech Republic. The consortium was obtained from a building surface in Brno in Czech Republic as a smear by a sterile swab and by the adhesive tape. The microorganism were cultivated into a BBM medium [5] at temperature 20 ± 3°C under light condition (2.5 kLux; with dark/light cycles 8 / 16 hour) during 14 days. They have been shaking by three times a day for 2 minutes. The aliquot from this culture was used for next experiments.

The microorganisms were isolated and after that it was identified. The consortium was composed from algae and bacteria. The bacteria were identified on basis their metabolic activity by use a commercial kit (Pliva-Lachema, Czech Republic) and by microscopy observation. The algae were identified by microscopy observation. There were identified following bacteria: *Burgohorderia cepacia* (0.8-1.0 x 1.6-3.2 μm, G^-), *Sphingomonas paucimobilis* (0.7 x 1.4 μm, G^-), *Sphingomonas multivorum* and these kinds of algae: *Aphanothece stagnina, Chroococcus bacillaris, Botrychloris cumuli*.

Preparation of nanofiber textiles

The tested nanofiber textiles were prepared by electrospinning technology at the Center for Nanotechnology in Civil Engineering at the Czech Technical University in Prague. There was used polyvinyl alcohol as a basic polymer in these experiments. The basic polymer solution for electrospinning was composed of 375 g 16% PVA (16R Sloviol, Fichema, Czech Republic), 3 g 85% H_3PO_4 (P-lab, Czech Republic) and 4.4 g of Glyoxal (Merck, USA). The biocidal nanofiber textileswere prepared with addition of supplement ions of copper ($CuSO_4{\cdot}5H_2O$), nanoparticle of diamond (with a diameter of 5 nm) and commercial biocide Lignofix E-Profi (Stachema, Czech Republic). The samples are specified in **Table 1**.

The nanofiber textiles were prepared by using a cylinder rotating electrode on device Nanospider LB 500 (Elmarco, Czech Republic) on spunbond textiles, which was prepared from polypropylene with antistatic properties. The setup of device was: rotation of the electrode was 10 Hz, a fabric speed was 5 Hz and a voltage was setup on 81.3 kV. The conditions during spinning were: relative humidity 40 %; temperature 27 °C.

All the nanofiber textiles were prepared as a single layer membrane. The nanofiber textiles were subsequently stabilized by heat for 10 minutes at 140 °C [6]. The each aliquot of a basic polymer solution was kept for the other experiment to evaluate efficiency of biocidal agents incorporated into the nanofiber textiles.

Table 1 Prepared samples of nanofiber textile and concentration of supplement in electrospinning polymer solution

Name of sample	Added supplement	Concentration of supplement in nanofiber textile (wt %)
PVA	None	0
PVA-Cu	Ions of copper ($CuSO_4{\cdot}5H_2O$)	1
PVA-ND	Nanoparticles of diamond	1
PVA-Bi	Biocide (Lignofix E-Profi)	1

Algae essay of biocide efficiency polymer solution

The consortium of algae and bacteria had been inoculated into 20 ml of the BBM medium in volume 5 ml (7 days incubation basic consortium). The biocide agents had been added into the solution composed of the BBM medium, microorganism and samples. The samples were 1 ml of each polymer basic solution (**Table 1**) or nanofiber textiles from same solution, which had 50 mm in a diameter and had immersed into the medium BBM with consortium. The growth of consortium was under light condition and the temperature was 20 ± 2 °C and it was shaken three times every day for 2 minutes. The evaluation of biocide properties was by measuring optical density in range from 200 to 800 nm, which correspond to the growth of microorganism.

Algae essay of biocide efficiency nanofiber textiles

The consortium of algae and bacteria had been inoculated on the BBM agar into the Petri dishes in volume 500 µl. The samples (**Table 1**) had cut from nanofiber textiles as a a circular sample with 20 mm in a diameter. The experiment was monitored for 14 days after inoculation and the evaluation were made by measuring of halo effects around the nanofiber textile.

3. RESULTS AND DISCUSION

Evaluation of anti-algae properties of nanofiber textiles are conducted by determination of concentration of microorganism in liquid media or as a halo effect around the sample on nutrient agar plate. The algae are coexisted together with bacteria and cooperated and therefore we measured whole absorbance spectrum (200 - 800 nm). The concentration is proportional to optical density. There are two significant peaks; the first one is around 300 nm and second one is around 635 nm.

The evaluation of biocidal properties nanofiber textiles on consortium algae and bacteria were measured by changing growth curves in significant peak. The first peak is around 300 nm and second one is around 635 nm. There is shown changing concentration of carbon in the peak of 300 nm [7] and it is evaluated as a change of the concentration of the bacteria and algae together. The alone algae have two significant peaks, the first one is around 480 nm and it is peak of change chlorophyll b and second one is around 680 nm and it is the peak of chlorophyll a [8]. The peak around 480 nm is hidden between other metabolic substances and it is unusable for this measuring. The peak of chlorophyll a is moved back into 635 nm for used consortium.

The nanofiber textiles treatment have same nanostructure after heat as before this type of stabilization. This stabilization can develop problem with biocide treatment which is degraded in upper temperature as a commercial biocide Lignofix E-Profi. The other way of stabilization is by using methanol, which damages nanofiber structure. The nanofibers stabilized by methanol are changing their diameter and the textiles look like membrane and the last way is by using of guanidine aldehyde [9]. The last mentioned method made insoluble nanofiber. The results are shown in graph below.

Table 2 Size of halo on the Petri dishes with consortium covered by a sample of a nanofiber textile

Name of sample	Size of halo around sample (mm)	Biofilm on nanofiber textile
PVA	0	strong growth
PVA-Cu	0.80 - 1.20	without growth
PVA-ND	0.05 - 0.10	weak growth
PVA-Bi	0.90 - 1.50	without growth

Alone PVA has not any biocide properties, phosphoric acid has only change pH of a polymer solution, but glyoxal has some biocide properties. In literature [10-11] are reported biocide properties of glyoxal from concentration 38 mmol / l. The efficiency against algae is stated from 149 mmol / l [10]. The influence on growth of bacteria is sited in range 40 - 400 mmol / l and concrete situation is following: the concentration in range 46-339 mmol / l causes inhibition of cell dividing [11], the concentration from 500 mmol / l inhibits cell breathing [12]. The prepared nanofiber textiles have up to 7.3 wt % of glyoxal in nanofibers and after recalculation it means that the concentration is up to 1.25 mmol / g. The samples used in experiment were in range from 20 to 50 mm and weight per square meter was 3.1 g / m^2 and it emerged, that we used up to 0.620 mmol in the presented experiments. This used value is under toxic limit for bacteria and algae and it is corresponding with no biocide properties of the PVA solution and the PVA nanofiber textiles.

The next nanofiber textiles is based on PVA solution doped copper ion, the concentration of copper in nanofiber was 1 wt % [13-14]. Copper serves as an essential component in metabolic processes of algae, playing vital function in electron transport and various enzymes system (amine oxidase, cytochrome c oxidase) [15]. But on the other hand copper is one of the most toxic heavy ions for algae and it is potential inhibitor of photosynthesis [16-17]. A slight increase in a endogenous Cu^{2+} concentration could interfere with various metabolic pathway causing inhibition of photosynthesis and it leads to deleterious effects at physiological, biochemical and structural levels [18-22]. In literature is reported that range 0.46 - 3.17 mg / l Cu to cause 20 - 70 % inhibition of growth after 96 h [23]. The samples from nanofiber textiles PVA-Cu had incorporated approximately 24.18 mg of Cu^{2+} in a solution experiment and 9.672 mg Cu^{2+} on an agar plate experiment, the result showed slight leaching effect on an agar plate and it is corresponding with stadium of culture in a stationary stage. The experiment with 1 ml of solution of PVA-Cu is corresponding with 50 mg / l of Cu^{2+}, the result showed decrease of growth algae and bacteria during first three day, but after it the copper was consumed or accumulated and the concentration of microorganism became growth. The 72 hour toxic limit is depending on composed of consortium [15].

The commercial biocide Lignofix E-Profi is composed of quarter ammonium salt, tebukonazol, propikonazol and so on. This solution is high toxic for water organism as algae [24]. The nanofiber textiles doped by these solutions are slowly leached from the nanofiber and keep concentration of biocide in the solution, same result is shown in the agar plate experiment.

Nanofiber textiles prepared origin from the PVA-ND solution had shown biocide activity until seven days, the same solution have high efficiency and it corresponded with the agar plate experiment, where is only a low halo effect. It is shown the strong binder between nanofiber and nanoparticles. Chithrani et al. reported that the reactivity of nanoparticles is depending on the size of particles more than on their concentration [25]. The size of nanoparticles is influenced by the type of preparation [26]. The nanoparticle of diamond interacted with cell wall and damaged by this way the cell [27].

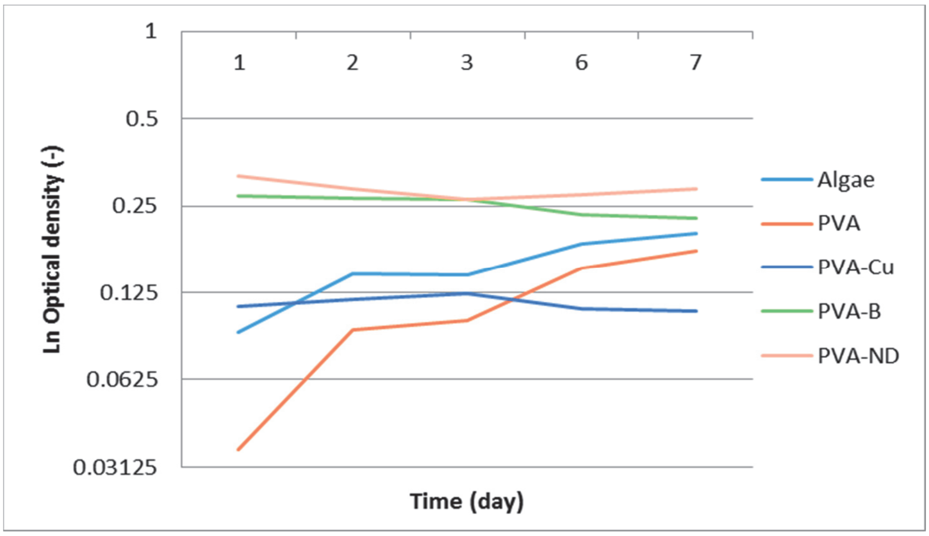

Fig. 1 Optical density at 298 nm, the curve are equivalent as a growth algae and bacteria together

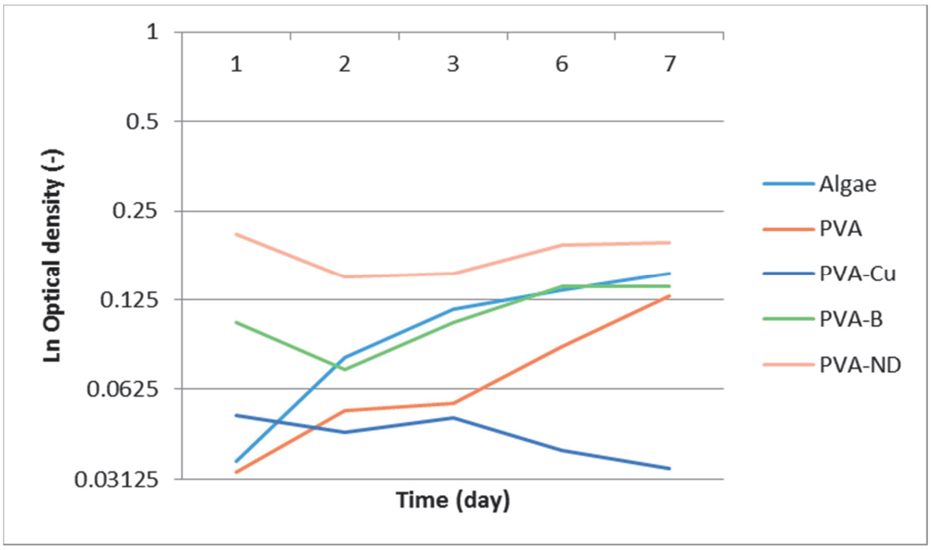

Fig. 2 Optical density at 635 nm, the curve are equivalent as a growth algae

4. CONCLUSION

We prepared nanofiber textiles doped biocide solution which has biocidal properties in first day in the solution, same results were shown in plate experiments. These properties are depending on used concentration of biocide and on consecutive leaching of it. This way of treatment is one possibility how to protect facades against biodegradation in the future.

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REFERENCES

[1] ESCADEILLAS, G., BERTRON, A., RINGOT, E., BLANC, P.J., DUBOSC, A. Accelerated testing of biological stain growth on external concrete walls. Part 2: Quantification of growths. Mater. Struct. 42, 2009, pp. 937-945.

[2] RANJAN, S., DASGUPTA, N., CHAKRABORTY, A.R., Melvin Samuel, S., Ramalingam, C., Shanker, R., Kumar, A. Nanoscience and nanotechnologies in food industries: opportunities and research trends. J. Nanopart. Res. 16., 2014.

[3] GAO, Y., BACH TRUONG, Y., ZHU, Y., LOUIS KYRATZIS, I. Electrospun antibacterial nanofibers: Production, activity, and in vivo applications. J Appl Polym Sci. 2014.

[4] LI, J., ZHOU, J., XU, Q. Progress of electrospun nanofiber scaffolds for tissue engineering. Chin. J. Tissue Eng. Res. 16, 2013, pp. 8847-8852.

[5] BISCHOFF, H.W., BOLD, H.C. Phycological Studies. IV. Some soil algae from Enchanted Rock and related algal species. - Univ. Texas Publ., 1963.

[6] TESÁREK, P., RYPAROVÁ, P., RÁCOVÁ, Z., KRÁLÍK, V., NEMECEK, J., KROMKA, A., NEŽERKA, V. Mechanical properties of single and double-layered PVA nanofibers. Key Eng Mat 586, 2014, pp. 261-264.

[7] SUTHERLAND, J.C., GRIFFIN, K.P. Absorption spectrum of DNA for wavelengths greater than 300 nm. Radiat. Res. 86, 1981, pp. 399-409.

[8] ROLDÁN, M., THOMAS, F., CASTEL, S., QUESADA, A., HERNÁNDEZ-MARINÉ, M. Noninvasive pigment identification in single cells from living phototrophic biofilms by confocal imaging spectrofluorometry. Appl. Environ. Microbiol. 70, 2004, pp. 3745-3750.

[9] ANDRADY, A.L. Science and Technology of Polymer Nanofibers. Sci. and Technol. Of Polymer Nanofibers, 2007, pp. 1-403.

[10] BOLLMAN, M.A., BAUNE, W.K., SMITH, S., DEWHITT, K. AND KAPUSTKA, L. Report on algal toxicity tests on selected Office of Toxic Substances(OTS) chemicals, 1989.

[11] HOECHST, A. Untersuchung auf Bakterienschädlichkeit: Zellvermehrungs-Hemmtest. Frankfurt, Hoechst AG, 1989, 1.

[12] GERIKE, P., GODE, P. The biodegradability and inhibitory threshold concentration of some disinfectants. Chemosphere 21, 1990, pp. 799-812.

[13] RACOVA, Z., RYPAROVA, P., HLAVAC, R., TESAREK, P., NEZERKA, V. Influence of Copper Ions on Mechanical Properties of PVA-Based Nanofiber Textiles. Experimental Stress Analysis 51 486, 2014, pp. 201-204.

[14] RYPAROVA, P., RACOVA, Z., TESAREK, P., WASSERBAUER, R. The Antibacterial Activity of Nanofiber Based on Poly-Vinyl-Alcohol (Pva) Doped by Metal, 2012, TANGER LTD, SLEZSKA; KELTICKOVA 62, SLEZSKA, OSTRAVA 710 00, CZECH REPUBLIC, pp. 23 - 25.

[15] KUMAR, K.S., DAHMS, H., LEE, J., KIM, H.C., LEE, W.C., SHIN, K. Algal photosynthetic responses to toxic metals and herbicides assessed by chlorophyll a fluorescence. Ecotoxicol. Environ. Saf. 104, 2014, pp. 51-71.

[16] GLEDHILL, M., NIMMO, M., HILL, S., BROWN, M. The toxicity of copper(II) species to marine algae, with particular reference to macroalgae. J. Phycol. 33, 1997, pp. 2-11.

[17] JANIK, E., MAKSYMIEC, W., MAZUR, R., GARSTKA, M., GRUSZECKI, W.I. Structural and Functional Modifications of the Major Light-Harvesting Complex II in Cadmium- or Copper-Treated Secale cereale. Plant Cell Physiol. 51, 2010, pp. 1330-1340.

[18] DUBEY, R. & MISHRA, S. Heavy Metal Toxicity Induced Alterations in Photosynthetic Metabolism in Plants. Books in Soils, Plants, and the Environment, 2005.

[19] KUMAR, K.S., HAN, Y.-., CHOO, K.-., KONG, J.-., HAN, T. Chlorophyll fluorescence based copper toxicity assessment of two algal species. Toxicol. Environ. Health Sci. 1, 2009, pp. 17-23.

[20] ZHANG, W., TAN, N.G.J. & LI, S.F.Y. NMR-based metabolomics and LC-MS/MS quantification reveal metal-specific tolerance and redox homeostasis in Chlorella vulgaris. Mol. BioSyst., 10(1), 2014, 149.

[21] HOOK, S.E., OSBORN, H.L., GISSI, F., MONCUQUET, P., TWINE, N.A., WILKINS, M.R., ADAMS, M.S.: RNA-Seq analysis of the toxicant-induced transcriptome of the marine diatom, Ceratoneis closterium. Marine Genomics. 2013.

[22] KUPPER, H., SETLIK, I., SPILLER, M., KUPPER, F., PRASIL, O. Heavy metal-induced inhibition of photosynthesis: Targets of in vivo heavy metal chlorophyll formation. J. Phycol. 38, 2002, pp. 429-441.

[23] XIA JIANRONG, TIAN QIRAN. Early stage toxicity of excess copper to photosystem II Chlorella pyrenoidosa-OJIP chlorophyll a fluorescence analysis. J. Environ. Sci. 21, 2009, pp. 1569-1574.

[24] KACMÁR, P., PISTL, J., MIKULA, I. Immunotoxicology and veterinary medicine. Acta Vet. Brno 68, 1999, pp. 57-79.

[25] CHITHRANI, B., GHAZANI, A., CHAN, W. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Lett. 6, 2006, pp. 662-668.

[26] DEMO, P., KOŽÍŠEK, Z., ŠÁŠIK, R.: Analytical approach to time lag in binary nucleation. Phys Rev E. 59, 1999, pp. 5124-5127.

[27] CHWALIBOG, A., SAWOSZ, E., HOTOWY, A., SZELIGA, J., MITURA, S., MITURA, K., GRODZIK, M., ORLOWSKI, P., SOKOLOWSKA, A. Visualization of interaction between inorganic nanoparticles and bacteria or fungi. Int. J. Nanomed. 5, 2010, pp. 1085-1094.