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INACTIVATION OF MICROORGANISMS IN MODEL BIOFILMS BY NON-THERMAL PLASMA AT ATMOSPHERIC PRESSURE

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One of the characteristics of microorganisms is their high ability to attach strongly to any surface and form biofilms. Biofouling is a widespread phenomenon in the nature and enginery. In the latter case the biofouling can lead to mechanical injury or biodamage. Indeed, given the needed culture medium, biofilms can form sizable mucilaginous layers diminishing drastically, for instance, pipe capacity. Besides, biofilms provoke intensively corrosion of metallic constructions and decrease essentially the lifetime of wood and concrete facilities.

The foregoing proves clearly that protection of industrial facilities, devices, materials, etc against the biofouling and biodamage (including biocorrosion) is one of the great challenges of a modern science and industry. This problem is rather difficult to resolve since biofilm-forming microorganisms are highly resistant to biocides. For instance, sterilization of the waterworks requires too high dose of biocides that is harmful from environmental point of view. Additionally, water treatment by biocides takes too long time (more than twenty-four hours).

Serious drawbacks of the traditional methods for microorganism inactivation and biofilm destroying stimulate a development of novel approaches to the protection against biofouling and biodamage processes. Here, we are dealing with so-called non-thermal plasma (NTP) sterilization of microorganisms including biofilms. NTP sterilization can lead to the eventual abandonment in usage of the heat and chemically aggressive, toxic and environmentally harmful liquid and gaseous agents. Our previous promising results on this topic are published in [1]. Present report contains new results on atmospheric pressure NTP inactivation of microorganisms in model (monoculture of *Escherichia coli* and *Bacillus subtilis*) biofilms.

Biofilms are spatially and metabolically structured microbial communities within extracellular polymeric matrix at the phase interface. Biofilms are characterized by the specific dynamics of the growth and substrate digestion modes, and are highly resistant to chemical biocides. A simple and convenient research model of a biofilm is pure culture colonies grown on agar. In the research we used biofilms generated by *E. coli* and *B. subtilis* monocultures.

The total number of microorganisms constituting *E.coli* biofilms grown in enriched medium reached 10^{11} CFU as early as overnight. As biofilms were getting old, the number did not change. But the biofilms increased in diameter, while their superficial density decreased (from 2.9×10^{9} CFU/mm^2 for a biofilm grown overnight to 13.6×10^{8} CFU/mm^2 for a three days old biofilm). After treating the biofilms with plasma, the biofilm grown overnight was found to be most susceptible, whereas the biofilm grown for three days was less susceptible. On the one hand the decreased susceptibility is likely associated with physical "ageing" of biofilm microorganisms. In this case cell division inhibits, whereas a pool of reserve cellular substances, as well as strength of the cell wall, increases. On the other hand, according to reported data old biofilms may contain a significant amount of dead cells to screen living cells against plasma particles.

Biofilms grown on the surface of agar media are convenient to work with, but they are only simplified models of naturally occurring biofilms. Biofilms grown on the surface of inert carriers are models that are close to natural biofilms. Biofilms for these experiments were grown either on mild steel coupons or on polypropylene coupons in tubes containing starvation medium 8E. Inoculates were *E. coli* and *B.subtilis* monocultures.

Treating *E.coli* metal and plastic coupons with low-temperature plasma showed full inactivation of microorganisms of biofilms, with the majority of cells being killed within the first minute of the treatment (Fig.1). The biofilm grown for 3 days on a metal coupon appeared to be the least susceptible.

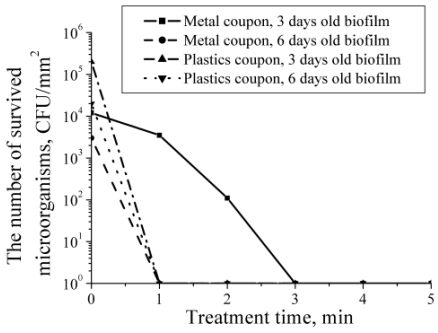

Fig.1. The rate of survived *E.coli* age-varying biofilms grown on metal and plastic coupons.

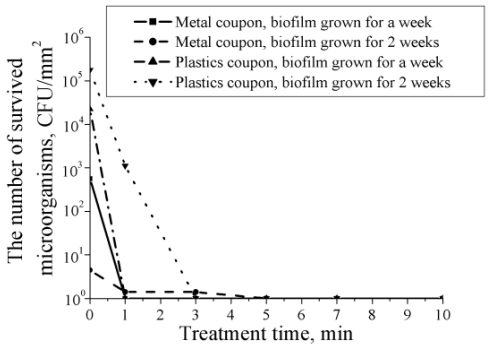

Fig.2. The rate of survived *B.subtilis* age-varying biofilms grown on metal and plastic coupons.

Research on *B.subtilis* biofilms grown on both types of coupons has shown that these biofilms are more tolerant than *E.coli* biofilms (Fig.2). Although the initial titer of *B.subtilis* biofilms was lower than that of *E. coli* biofilms, they became inactivated in 3-5 minutes of the treatment. Besides, in contrast to *E.coli* biofilms, «green» (one week old) *B. subtilis* biofilms appeared more susceptible than two weeks old biofilms, probably due to the transition of some *B. subtilis* cells from vegetation to spores.

Obtained results demonstrate a great potential of non-thermal plasma at atmospheric pressure as a tool to inactivate various biofilms. The degree of inactivation is shown to depend strongly on the composition of the nutrient medium. The type of the surface also influences much the survival of biofilm microorganisms. *E.coli* biofilms grown on polypropylene coupons were most susceptible, but they became tolerant when cultured on the enriched agar medium.

Results obtained allow us to conclude that the plasma sterilization procedure differs beneficially from conventional methods to control biofilms. No chemically aggressive reagents are required, and the plasma procedure takes short time. Owing these specific features the procedure is expected to be widely applied in different areas where control and inhibition of the growth of biofilms are urgent (pipelines, surfaces of stone, wood and concrete buildings, monuments).

Reference

[1] Yu. Akishev, M. Grushin, V. Karal'nik, A. Petryakov, N. Trushkin, V. Kholodenko, V. Chugunov, I. Irkhina, E. Kobzev, N. Zhirkova and G. Kireev 2008 *Pure and Applied Chemistry* **80** 1953.