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DEGRADATION OF BACTERIA *ESCHERICHIA COLI* BY TREATMENT WITH Ar ION BEAM AND NEUTRAL OXYGEN ATOMS

UNIČEVANJE BAKTERIJ *ESCHERICHIA COLI* S CURKOM IONOV Ar IN NEVTRALNIH ATOMOV KISIKA

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Scanning electron microscopy was used to determine the difference between bacteria degradation by two types of particles presented in gaseous plasma, i.e. positively charged ions and neutral oxygen atoms. The source of ions was an argon ion gun with the ion energy of 1 keV and the flux of 3×10^{18} m^{-2} s^{-1}. The source of neutral oxygen atoms was inductively coupled oxygen plasma supplying the flux of oxygen atoms of about 1.5×10^{23} m^{-2} s^{-1}. The ion beam treatment time was 1800 s while the oxygen atom treatment time was 300 s. Bacteria *Escherichia coli,* strain ATCC 25922 were deposited onto well activated aluminum at the concentration of about 3×10^6 cfu and exposed to both particles. SEM analysis was performed using a field emission microscope with the energy of primary electrons of 1 keV. SEM images revealed huge difference in morphology of bacteria treated by both methods. While ions tend to drill holes into bacterial cell wall, the atoms caused a more even disruption of bacterial cell wall. The results were explained by kinetic, potential and charging effects.

Key words: bacteria, *Escherichia coli,* sterilization, degradation, oxygen plasma, atoms, ions, SEM

Z vrstično elektronsko mikroskopijo smo raziskovali razliko v degradaciji bakterij pri obdelavi z dvema različnima vrstama delcev v plinski plazmi: s pozitivno nabitimi in z nevtralnimi kisikovimi atomi. Vir ionov argona z energijo 1 keV in tokom 3×10^{18} m^{-2} s^{-1} je bila ionska puška. Vir nevtralnih atomov kisika s tokom $1{,}5 \times 10^{23}$ m^{-2} s^{-1} na površino vzorcev pa je bila induktivno sklopljena kisikova plazma. Čas obdelave z ioni je bil 3000 s, medtem ko je bil čas obdelave s kisikovimi atomi 300 s. Bakterije *Escherichia coli*, sev ATCC 25922 smo nanesli na dobro aktivirano površino aluminija in jih potem izpostavili curkom obeh vrst delcev. Koncentracija bakterij je bila 3×10^6 cfu. Po obdelavi smo površino vzorcev analizirali z vrstično elektronsko mikroskopijo (SEM). SEM-slike so razkrile veliko razliko v morfologiji bakterij, obdelanih z atomi oziroma ioni. Medtem ko ioni povzročijo nastanek lukenj v celični steni bakterij, pa atomi bolj enakomerno degradacijo celične stene. Dobljene rezultate smo razložili z vplivom kinetičnih in potencialnih efektov ter vplivom nabijanja površine.

Ključne besede: bakterije, *Escherichia coli*, sterilizacija, degradacija, kisikova plazma, atomi, ioni, SEM

1 INTRODUCTION

Plasma sterilization has attracted much attention in the past decade due to possible application for sterilization of delicate materials that cannot stand autoclaving in humid air at 130 °C. Several different types of discharges have been used to create plasma suitable for destruction of vital bacteria and their spores.[1–9] The discharges include low and atmospheric pressure. Among atmospheric discharges, RF and microwave plasma torches are particularly popular, while the dielectric barrier glow discharge was not found as efficient. The same applies also for otherwise popular corona discharges. The low pressure discharges suitable for destruction of bacteria at low temperature include the DC, RF and microwave discharges.[10–14] Radiofrequency discharges are particularly popular since they assure for a high density of plasma radicals and rather low kinetic temperature of neutral gas.

Most authors presented results on bacterial deactivation as a function of discharge parameters. The discharge parameters that are often varied include the type of gas or gas mixture, the pressure in the discharge tube and the gas flow, the discharge power, the dimensions and the type of material used for the discharge chamber, etc. Much less work, however, has been done on determination of sterilization effects versus plasma parameters. Not surprisingly, the explanations of observed sterilization effects are often contradictory. Many authors explain sterilization by destruction of bacterial DNA caused by UV photons from plasma. Other authors state that sterilization is due to chemical etching of the bacterial cell wall with radicals such as O, N, H, etc. Some other authors take into account also the kinetic effects of bombardment with positive ions, and most authors agree that synergetic effects play an important role.

In order to understand the role of different plasma particles it is the best to separate them and treat bacteria only with one type plasma particles. At the experiments presented in this paper we exposed bacteria separately to 2 types of different plasma particles: energetic non-reactive ions and neutral oxygen atoms with the kinetic temperature of 300 K.

2 EXPERIMENTAL

2.1 Sample preparation

Bacteria *Escherichia coli* (*E. coli*) were cultivated according to the standard procedure. In experiment we used bacteria *E. coli* strain ATCC 25922. It was grown at 37 °C, on LB plates for 24 h. Cells were then resuspended in sterile water. Number of cells was adjusted to approximately 3×10^6 cfu (colony forming unites).

Live bacteria were deposited onto commercially available aluminum foils. Substrates were first carefully cleaned with wet chemical treatment, and then activated with a brief exposure to oxygen plasma in order to assure the removal of any traces of organic contaminants and achieve optimal hydrophilicity. A drop of water containing vital bacteria was placed onto the substrate. Due to highly activated surface, the bacteria-containing water drop was spread on a large surface. Such spreading allowed for two dimensional distributions of bacteria with out overlapping.

2.2 Experimental system

Samples were treated either by neutral oxygen atoms in an afterglow chamber of oxygen plasma reactor or by positively charged Ar ions from a commercial ion gun. The schematic of the experimental setup for the case of oxygen atoms is shown in **Figure 1**. The vacuum system is pumped with a two stage rotary pump. The effective pumping speed at the exit of the experimental chamber is almost identical to the nominal pumping speed of the pump, i.e.16 m^3/h. The experimental chamber is connected to a discharge chamber through a narrow tube that allows for a difference in the effective pumping speeds between the experimental and discharge chambers and thus a pretty high drift velocity of gas through the narrow tube. Both chambers as well as the connection tube are made from borosilicate glass Schott 8250. This glass has a low recombination coefficient for the reaction $O + O \rightarrow O_2$.[15,16] Such a configuration assures for experiments at constant (i.e. room) temperature and constant density of oxygen atoms in the vicinity of substrates. The density of neutral oxygen atoms is measured with a catalytic probe.[17-19] At the experimental pressure of 75 Pa the O density is about 1×10^{21} m^{-3}. The resultant flux of neutral oxygen atoms onto the surface of the sample is then $j = ¼\ nv = 1.5 \times 10^{23}$ m^{-2} s^{-1}.

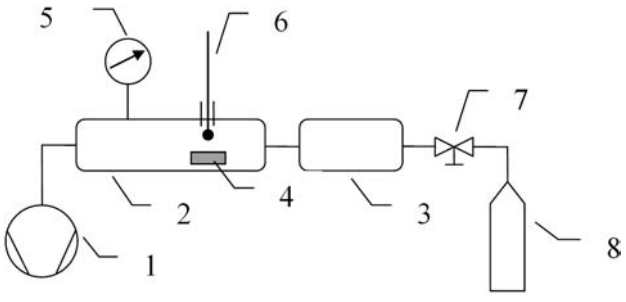

Figure 1: The experimental setup for treatment of bacteria with neutral oxygen atoms. 1 – vacuum pump, 2 – experimental chamber, 3 – discharge chamber, 4 – sample, 5 – vacuum gauge, 6 – catalytic probe, 7 – inlet valve, 8 – oxygen flask

Slika 1: Shema eksperimentalnega sistema za obdelavo bakterij z nevtralnimi atomi kisika: 1 – vakuumska črpalka, 2 – eksperimentalna komora, 3 – razelektritvena komora, 4 – vzorec, 5 – vakuummeter, 6 – katalitična sonda, 7 – dozirni ventil, 8 – jeklenka s kisikom

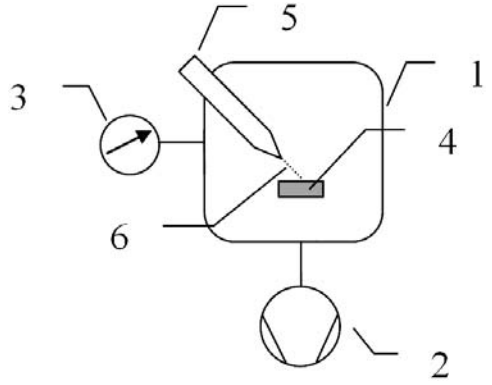

Figure 2: The experimental setup for treatment of bacteria with Ar ions: 1 – UHV chamber, 2 – pumping system, 3 – vacuum gauge, 4 – sample, 5 – ion gun, 6 – energetic ions.

Slika 2: Shema eksperimentalnega sistema za obdelavo bakterij z ioni Ar: 1 – UVV komora, 2 – črpalni sistem, 3 – vakuummeter, 4 – vzorec, 5 – ionska puška, 6 – energijski ioni

The experimental setup for treatment of bacteria with Ar ions is shown schematically in **Figure 2**. The source of Ar ions is a commercial ion gun used for sputtering of materials during depth profiling. Ar ion beam with the energy of 1 keV at an incidence angle of 45° and a raster of 3 mm × 3 mm was used for treating bacteria. The ion current is 0.15 A/m^2 giving the ion flux onto the surface of the substrate with bacteria of 3×10^{18} m^{-2} s^{-1}. We used no charge compensation during treatment of bacteria with argon ions.

2.3 SEM imaging

Scanning electron micrographs of substrates with bacteria were obtained using a field emission microscope Karl Zeiss Supra 35 VP. A 1 kV accelerating voltage was used to record images.

3 RESULTS

SEM image of untreated *E. coli* bacteria is shown in **Figure 3**. The image does not look very sharp. This is not an artifact of the microscope but rather the consequence of the presence of the capsule on the surface of bacteria as well as between bacteria. Namely, the capsule is composed predominantly of chemically bonded water as well as some sugars, proteins and lipids – material that are a bad scatterer for electrons. That's why the SEM image looks rather dim.

A SEM image of a bacteria treated by Ar ions is shown in **Figure 4**. The bacteria are badly damaged and definitely not capable of revitalization.

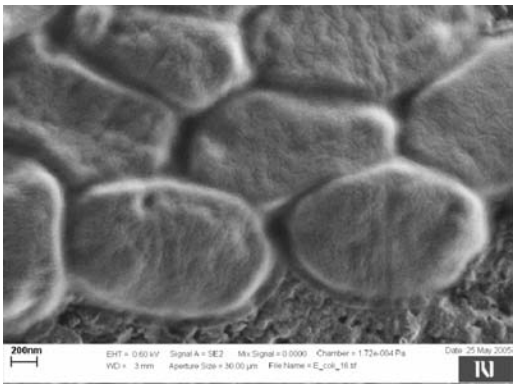

Figure 3: SEM image of untreated bacteria
Slika 3: SEM-slika neobdelane bakterije

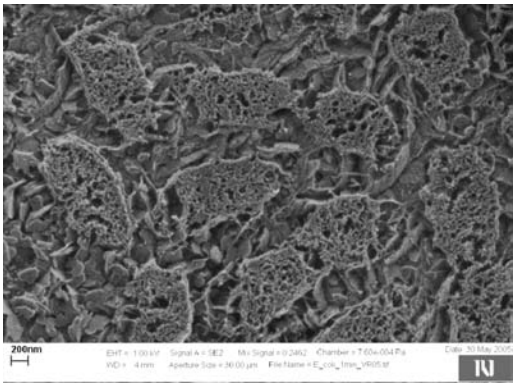

Figure 5: SEM image of bacteria treated with oxygen atoms
Slika 5: SEM-slika bakterije, obdelane z atomi kisika

A SEM image of bacteria treated in the afterglow of the oxygen plasma, i.e. with neutral oxygen atoms only, is presented in **Figure 5**. In this case, the surface morphology is very different from that observed in **Figure 4**.

4 DISCUSSION

Figures 3, 4 and **5** represent SEM images of bacteria *E. coli*. Bacteria presented in **Figure 3** are live what has been confirmed by cultivation using the standard plate count technique. Bacteria are covered with a thin film of jelly of lipopolysaccharides and is called capsule. The majority of lipopolysaccharide cover material has chemically bonded water. This thin cover is (about 400 nm or more) capsular polysaccharide gel[20] which serves as a medium for gluing bacteria together as well as for sticking onto surfaces. The capsule also facilitates formation of three dimensional clusters of bacteria. Such clustering was not observed at our experiments since we activated the surface of the aluminum prior to bacterial deposition. The surface of activated aluminum foil is perfectly hydrophilic thus allowing for two- dimensional spreading of bacteria on its surface. Such procedure for bacteria fixation therefore allows for uniform treatment of bacteria with plasma particles.

An exposure of bacteria to argon ions causes a strong damage. **Figure 4** represents the SEM image of bacteria

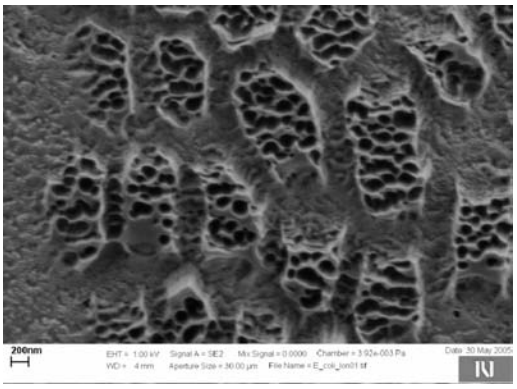

Figure 4: SEM image of bacteria treated with argon ions
Slika 4: SEM-slika bakterije, obdelane z ioni argona

after receiving the argon ion dose of 5.4×10^{21} m^{-2}. The bacteria are definitely not capable of revitalization what was proved also by control experiments using the plate count technique. It is interesting that the damage caused by ions is far from being uniform. Namely, a hole – like structure of the bacterial cells is observed. Although it is known that ion beam etching is never perfectly homogeneous and isotropic, such rich surface morphology cannot be due to common effects observed at ion beam etching of organic materials. The observed morphology may be attributed to appearance of the local surface electrical charge during treatment with positively charged ions. Namely, the electrical conductivity of bacteria is poor. Since the composition of the cell wall is far from being uniform, some spots on the surface may keep larger charge than other. The surface charge influence the local uniformity of the ion flux on the surface causing local focusing and thus further non-uniformity of the ion beam etching. Finally, the bacteria obtain morphology as shown in **Figure 4**. The ions practically cannot reach the uppermost part of bacteria since positive charge prevents it.

The SEM image of bacteria treated with oxygen atoms shows a completely different picture. In this case, the badly damaged bacteria are flattened, also. In fact, little material remained after receiving the dose of approximately 4.5×10^{25} m^{-3}. The remains observed on the surface of the aluminum foil after treatment with oxygen atoms represent only ash – mostly inorganic remains of the bacterial material after rather complete oxidation of organic material. This picture is in agreement with previous observations on selective etching of organic materials by oxygen radicals[21].

5 CONCLUSIONS

Bacteria *E. coli* were deposited onto aluminum foils and exposed to positively charged argon ions or neutral oxygen atoms in the ground state. In both cases, the samples were kept at room temperature. Since argon is inert gas that does not interact chemically with organic material, the interaction was almost completely kinetic. Apart

from radiation damage, the argon ions caused sputtering of the bacterial material. The sputtering was extremely inhomogeneous what was explained by local charging of the bacteria. In the case of oxygen atoms, any kinetic effect is neglected since the O atoms are thermal at room temperature. In this case, rather uniform degradation of bacteria occurred and only ashes remained after the treatment. The interaction of O atoms with bacteria is therefore purely chemical. In both cases, bacteria were badly damaged and unable to revitalize.

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