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Research Article

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Does Electrochemically Reduced Water Remove Bacterial Film?

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ABSTRACT

This work aimed to study the structure of bacterial film grown on the inner tube surface of the flow reactor. Applying scanning electron microscopy (SEM) approaches, the detailed biofilm relief was visualized. The action of electrochemically aqueous solution on the fine structure of biofilms generated by plankton forms of lacto bacteria and E.coli was investigated. Electrochemically aqueous solution treatments were able to destroy the biofilm organic polymer matrix and bacterial cells embedded in a matrix.
It is shown that the working of a bacterial film with alkaline catholyte destroys the main components of the biofilm, having a significant purifying and disinfecting effect. One of the criteria for the effectiveness of biofilm purification is the morphological analysis of the presence of fragments of the matrix or cellular component on the specimen surface. By the SEM method, the presence of a residual organic mass on the surface of the substrate after treatment was observed. This is a factor that can provoke de novo formation of a population of microorganisms on the surface of the pipeline surface. Thus, the most important task of cleaning and disinfection of the pipeline is the removal of microbial cells and residual fragments of the biomatrix.

Key words: *Bacterial Film, Scanning Electron Microscopy, Flow Biofilm Reactor, E.Coli, Lactate Bacteria, Electrochemically Activated Water*

INTRODUCTION

In many branches of industry biofilm leads to significant loss of resources and reduced efficiency that causes to search for ways of its removal [1]. The microbial film is formed on a solid surface in a moist medium in the form of a multicellular community immersed in a polymer matrix [2-3]. In comparison with plankton form biofilm, forming its own homeostasis, provides microorganisms with protection from antibiotics, as well as from mechanical destruction by a liquid stream [4-6]. This situation requires a significant increase in the concentration of antimicrobial drugs, which exerts an additional burden of the environment. Therefore, it is important to develop fundamentally new ways of removing the biofilms that are economical, effective and environmentally safe. An alternative to conventional cleaning is surface treatment with electrochemically activated (ECA) aqueous solutions with a wide bactericidal range of action [7-8]. This biophysical approach is based on the fact that the ECA aqueous solution in a metastable state characterized by a change in the oxidation-reduction potential (ORP), and after a short time restores the original properties. The purpose of this work was to investigate the effect of the treatment of the bacterial film by ECA solutions on the cellular component and matrix.

MATERIALS AND METHODS

The work was performed on a bacterial film formed in a flow reactor under the conditions of a laboratory experiment [9-11]. A layer of microorganisms was formed on the inner surface of a porous polyvinylchloride (PVC) tube. The biofilm source was the plankton lactic acid bacteria (Lactococcus lactis, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus helveticus, Propionibacterium freudenreichii ssp. Shermanii) or bacteria and *E.coli* mixture. To remove the bacterial film, the inner cavity of the tube was treated with a stream of alkaline catholyte (pH 13.4÷13.5,

ORP -35mV ÷ -50 mV). ECA solution was obtained with the electrochemical plant STEL-ANK-SUPER (LLC "Delfin Aqua", Russia).

A bacterial film washed with ordinary water (pH 7.3÷7.6, ORP 250mV ÷ 290mV) was used as a control. The change in the fine structure of the sample was studied by scanning electron microscopy (SEM). The basic principles of preparation for SEM were described earlier [12-13]. Briefly, a segment of the recirculation reactor tube was fixed in a solution of 2.5% glutaraldehyde at 4° C for 12 hours, then in a solution of 0.5% OsO_4 at room temperature. After fixation, the sample was dehydrated in a battery of ethanol with increasing concentration: 50%, 75%, 80%, 90% and 98%. To remove alcohol, the samples were transferred to HMDS (hexamethyldisilazane), after which they were dried in the air. The finished preparation was attached to a microscope holder followed by platinum (10 nm) film coated on the sample surface in a JFC-1600 unit (JEOL, Japan). The fine structure of the surface relief was studied in a scanning electron microscope JSM-6390A (JEOL, Japan), using the secondary electron mode at an accelerating voltage of 10 kV.

RESULTS & DISCUSSION

The figure 1 shows SEM micrographs of the lactic acid bacteria film. The biofilm is formed on the inner surface of a porous PVC tube in a recirculating reactor under different conditions of removal of the bacterial layer.

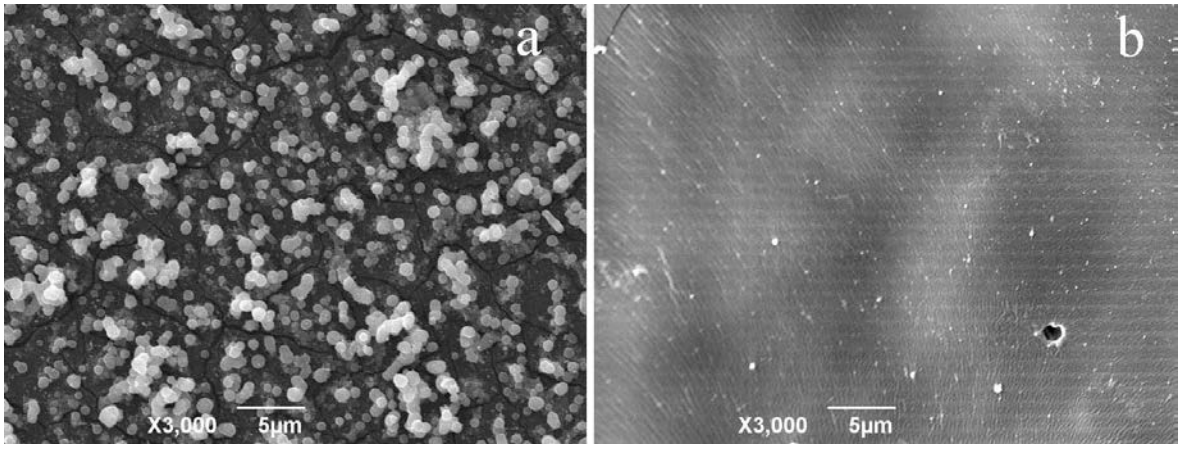

Figure 1. Micrographs of a bacterial film on the inner surface of a PVC tube

The images were obtained by scanning electron microscopy in the secondary electron mode:

(a) the film formed by the composition of lactic acid bacteria and washed with a flow of ordinary water;

(b) the film formed by the composition of lactic acid bacteria and washed by the flow of the ECA aqueous solution, catholyte.

From the data analysis (Fig.1), it can be concluded that the laboratory reactor used is effective for the formation of a bacterial film. The proposed procedure allows one to specify the experimental parameters and to simulate the biofilm generation followed by cleaning with ECA aqueous solution. Comparison of the biofilm fine structure after washing with ordinary water or catholyte exhibits significant differences. On the microphotography of the control sample (Fig.1a), it is impossible to distinguish the types of lactic acid bacteria, but a compactness of cellular multilayer indicates the formation of a mature matrix. A different event is observed after washing the tube lumen with catholyte (Fig. 1b) where there is the complete removal of cellular composition. Thus, catholyte has a significant purifying and disinfecting effect, although the degraded matrix fragments remain on the tube surface. These fragments are not visualized by optical microscopy and may cause the bacterial film rapid regeneration. It may be necessary to increase the treatment time and/or use the combined sequential action of the ECA fractions of the solution, characterized by an opposite in sign (high positive and negative) ORP value for more efficient removal of the matrix.

It is of interest to form and disintegrate a film of lactic acid bacteria formed in the presence of *E. coli.* This microorganism coexists in symbiosis with man and is easily spread in many spheres. Therefore, *E.coli* is a sanitary-indicative microorganism in food and agricultural industries. Its presence in the samples serves as a criterion for the unfavorable state of the enterprise. Comparative micrographs illustrating this experiment are shown in Fig. 2.

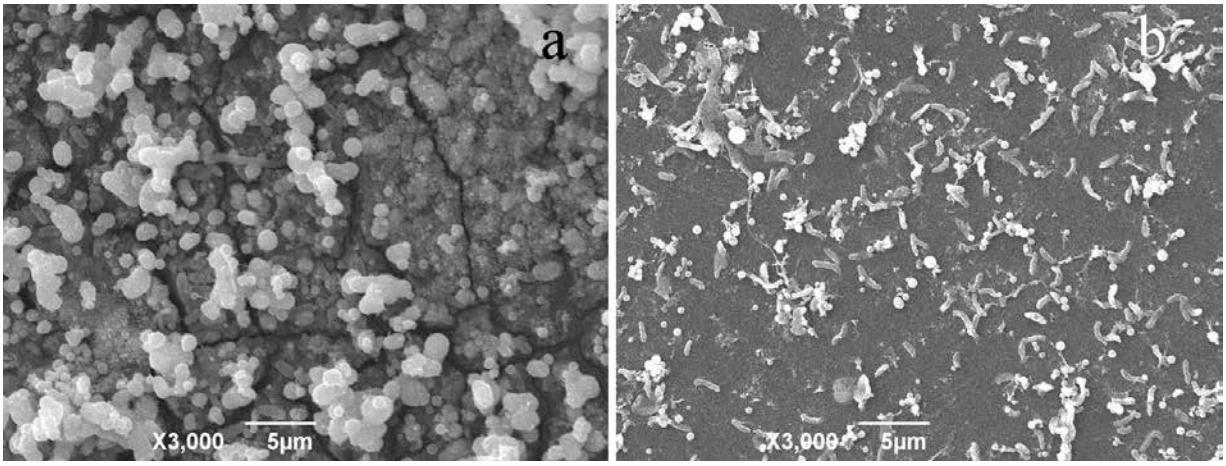

Figure 2. Micrographs of a bacterial film on the inner surface of a PVC tube

The images were obtained by scanning electron microscopy in the secondary electron mode:
(a) the film formed by the lactic acid bacteria and *E. coli* mixture and washed with a flow of ordinary water;
(b) the film formed by the lactic acid bacteria and *E. coli* mixture and washed in succession with a flow of ECA aqueous solution, catholyte
It can be seen (Fig. 2a) that the control sample is characterized by a structure in the form of cell colonies containing *E.coli*. As a result of processing the ECA succession with catholyte, the form of the film changes significantly (Fig. 2b). As in the case without *E.coli*, lactic acid bacteria are completely removed while matrix elements remain. The difference is that after the catholyte action, cellular fragments remained on the sample surface. The result of PCR-RT analysis (data not presented) showed that they belong to the *E. coli*. Perhaps this effect is explained by the fact that the adhesion of *E.coli* to the relatively soft surface of the PVC tube is due to not only adhesion, but also mechanical fastening by means of a filamentous appendage.

CONCLUSION

Taken together, we can draw the following conclusions. In laboratory conditions bacterial biofilms of lactic acid bacteria and E. coli were formed in the flow reactor on the inner surface of a porous tube made of polyvinylchloride. Treatment of a bacterial film by alkaline catholyte (pH 13.4÷13.5, ORP -35mV ÷ -50 mV) destroying the main components of the biofilm produces a significant purifying and disinfecting effect. One of the criteria for the effectiveness of biofilm purification is the morphological analysis of the presence of fragments of the matrix or cellular component on the specimen surface. By the SEM method, the presence of a residual organic mass on the surface of the substrate after treatment was observed. This is a factor that can provoke *de novo* formation of a population of microorganisms on the surface of the pipeline surface. Thus, the most important task of cleaning and disinfection of the pipeline is the maximum possible removal of microbial cells and residual fragments of the biomatrix.

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