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Polymeric materials – selected standards and biological research methods

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ABSTRACT

The purpose of this paper is to present the standards and the research methods enabling biological examination of polymeric materials or active packaging. These procedures apply to polymeric materials containing additives such as bactericides and fungicides.

***Keywords*:** polymer materials, biocide, biofilm, mutagenicity

1. INTRODUCTION

Polymeric materials are currently very commonly used. They occur in almost every branch of economy. The branch of economy that currently develops the fastest refers to the production of objects of everyday use. This is strictly related to the development of new technologies and innovative materials. Very often innovative materials concern intelligent materials and active packaging, therefore, they need to be studied not only in terms of physico-chemical, but also biological properties [1÷6].

A group of experts representing five Nordic countries: Denmark, Finland, Sweden, Norway and Island, associated within European Economic Agreement – FEA, developed the *'Nordic Report'*, providing in it the definition of active packaging. According to them, *"active packaging"* is a packaging, which apart from fulfilling a role of a barrier protecting from the impact of external factors, also serves additional functions, controlling or even affecting the course of phenomena occurring inside of the packaging" [2].

The available literature very often provides review publication informing of ways or methods of polymeric materials examination for the purpose of determining their physico-chemical properties or degradation [7÷10]. No publication that presented the possibilities of analysis of polymeric materials in terms of biological research was found. Therefore, it was decided to undertake work in this area. Thus, this article constitutes a review of literature related to the selected standards and biological examination methods of polymeric materials in different scientific centres.

Biological examinations of polymeric materials are important because microorganisms can settle on biotic or abiotic surfaces, including packaging and foils. The microorganisms adhesively stick to surfaces and create layers, called biological membranes or biofilm. It allows them to have an easier access to nutrients and also protects their cells from negative influence of environmental factors. The adhesive abilities of microorganisms have great influence on the ability to cause infections and contaminations [11÷12]. These cells adhere to one another and to solid surfaces. The bacterial cells that live in a biofilm environment show resistance to a significant part of antibacterial agents and are about 1000 times more resistant to these agents than microorganisms remaining in suspension [13].

The bacterial biofilm is a structure protecting cells from antibacterial agents. Apart from its protective function, it also plays a great role in initial phase of degradation. Contamination of polymeric materials is the beginning of degradation [14]. Material destruction processes, which occur under the action of biological activity is called biodeterioration or corrosion caused by microorganisms (*MIC – microbiologically influence corrosion*). The bacterial biofilm can be limited by introducing biocidal substances into the polymeric matrix. While raising the subject related to bactericidal or biostatic substances, two notions need to be explained: *"antibacterial activity"* and *"antibacterial efficiency"*, which are often incorrectly applied [26-30].

The antibacterial activity has specific numeric value. It is the difference in logarithms between living bacterial cells counted on a product (e.g. foil) containing antibacterial substance and the product free from this substance. On the other hand, the antibacterial efficiency is the ability of the antibacterial substance to inhibit growth of bacteria on polymeric material surface containing the bactericidal substance. The antibacterial efficiency is determined by antibacterial activity.

2. RESULT

The most recent scientific achievements described in the world literature explicitly indicate the development of biological research methods regarding polymeric materials. The examination of polymeric materials in biological context is difficult because of the diversity of biological factors, which additionally often can be "capricious". The results of the biological determinations can differ among themselves. It has an impact on, for example, the size of collected microbiological *inoculum*, temperature conditions, pH, type of a microorganism or times of incubation.

2. 1. Standards regarding the biological examinations

Below listed are the most important standards enabling the conduction of biological examinations of polymeric materials.

a) **PN-EN ISO 7218:2008/A1** entitled: "Microbiology of Food and Animal Feeding Stuffs. General Requirements and Guidance for Microbiological Examinations". The scope of the standard contains general instructions, regarding the conduction of microbiological examinations. The standard also refers to other standards, which provide credibility of the examinations. In the standard presented there are rules that are used in every laboratory, which ensures that the results obtained are unified.

b) **PN-EN ISO 846:2002** entitled: "Plastics. Evaluation of the Activity of Microorganisms". It is a qualitative evaluation of the influence of microorganisms on plastics in the result of a direct interaction – damaging the plastics which constitute the medium for microbial growth of microorganisms, and indirect interaction – i.e. the influence of microorganisms' metabolism products such as discoloration.

The scope of the standard involves the methods of determination of the impact of fungi (*Aspergillus niger, Penicillium funiculosum, Chaetomium globosum, Gliocladium virens, Paeliomyces variotii*), bacteria (*Pseudomonas aeruginosa*) as well as soil microorganisms on polymeric materials. These impacts are defined by: change of appearance, change of mass and other physical properties. These methods can be applied for examination of products with smooth surface.

c) **ASTM E2149-13a** entitled: "Standard Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions". It is a standard method of examining the antibacterial activity of an agent under dynamic contact condition.

d) **ISO 22196:2011** entitled: "Measurements of antibacterial activity on plastics and other non-porous surfaces". This standard refers to quantitative determination of bactericidal properties of foil. In the examinations two reference bacteria strains are used: *Staphylococcus aureus* (ATCC 6538P) and *Escherichia coli* (ATCC 8739). Antibacterial properties (R) of particular test foils are determined in relation to control sample, which does not contain any potentially bactericidal substance. According to the standard ISO 22196:2011 the foils are considered to demonstrate bactericidal properties if the reduction of bacteria cells that are able to grow is at least two orders of magnitude higher than on the control samples.

e) **ASTM G21-09** entitled: "Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi".

This standard describes 4-week examination of evaluation of material resistance to fungi and mould (*Aspergillus niger, Penicillium pinophilum, Chaetomium globusom, Gliocladium virens, Aureobasidium pullans*). The form of the result is visual assessment.

f) **PN-EN 15458:2014-09** entitled: "Paints and Varnishes – Laboratory Method for Testing the Efficacy of Film Preservatives in a Coating against Algae".

g) **PN-EN 15458:2014-10** entitled: "Paints and Varnishes – Laboratory Method for Testing the Efficacy of Film Preservatives in a Coating against Fungi".

Each of these standards suggests different approach to the research problem and different possibilities of analysing a specific case of a polymeric material. Apart from standards proposed by the Polish Committee for Standardisation (PKN), the American Society for Testing and Materials (ASTM) and the International Organisation for

Standardisation (ISO), there are methods or research procedures proposed by scientists, which are presented in scientific publications. Some of them are listed below, in point 2.2.

2. 2. Non-standardised methods of biological examination of polymeric materials

Below listed are the selected research methods enabling polymeric material examination in terms of their biological properties.

a) **Determination of bacterial biofilm created on polymeric materials**

The bacterial biofilm can be determined with the use of a spectrophotometer or a microscope, most often fluorencence or scanning electron microscope (SEM). The examination of bacterial biofilm can be conducted using spectrophotometric method of measuring the amount of crystal violet bound by bacteria cells creating biofilm [15÷17]. In this method arbitrary strains of bacteria can be used.

Bacterial cells with biofilm can also be analysed for their ability to survive with the use of epifluoroscence microscopy. In that case, the live/dead method is applied, by staining the living cells (green), and the dead cells (red) present on the polymeric material with fluorochromes [18].

The bacterial biofilm can also be determined using scanning electron microscopy, by taking pictures usually with magnification of (≥ 1 000x) [19].

b) **Determining the bactericide properties of polymeric materials**

The bactericide properties of polymeric materials with antibacterial additive can be determined using the contact-diffusion method on the pure cultures of indicative organisms. The examination consists in creating a “stamp” from a fragment of defined size of polymeric material onto the microorganism strained Petri dish and incubating the dish for specified amount of time, in specified temperature. After this time the intensity of the indicative bacteria growth is assessed in relation to reference samples [20].

c) **Determining the mutagenicity of polymeric materials**

Determining the mutagenicity of polymeric materials is based on *Ames test*. This test uses auxotrophic mutants of *Salmonella*. This type of bacteria is unable to grow in an environment deprived of histidine. If in the presence of, for example, antibacterial substance introduced to the polymeric material, the *Salmonella* cells are able to grow, that would mean that these materials are mutagenic [21].

d) **Determining the toxicity of polymeric materials**

Determining the toxicity of polymeric materials can be conducted on the basis of luminescence loss test in *Vibrio fisherii* strain in the presence of examined polymeric materials. *Vibrio fisheri* is a bacteria which demonstrates luminescence in favourable conditions. Under the influence of toxic substances the luminescence decreases. By comparing the luminescence of the strain in the presence of the specific and the control samples, the toxicity of the examined materials can be determined. The luminescence measurement is performed on a luminometer [22].

e) **Determining the phytotoxicity of polymeric materials**

Phytotoxicity examinations can be conducted with the use of seeds: spring barley (*Hordeum vulgare*), radish (*Raphanus sativus*) and garden cress (*Lepidum sativum*). The phytotoxicity examination consists in the inhibition or stimulation of the growth of selected above mentioned plants: in form of the number of particular organs, heights, stem lengths. The phytotoxicity examination can also consist in determining color changes, necrosis, chlorosis, deformation: in form of the number of damaged plants (or their parts), or the percentage of surface, on which the changes occur [7,23].

f) **Determining the influence of polymeric materials on the activity of extracellular bacterial hydrolases**

Determining the influence of polymeric materials on the activity of extracellular bacterial hydrolases is important from the perspective of biodegradation. This is because it is biodegradation, for example of polylactides (PLA), which results mainly from the activity of extracellular enzymes. General activity of hydrolytic enzymes after contacting the polymeric material is determined with the use of non-specific substrate - fluorescein diacetate [24]. The concentration of fluorescein released under the action of the hydrolases is measured with the use of a spectrofluorometer, respectively to the calibration curve, at the wavelength of 480 nm and emission wavelength of 505 nm. The inhibition or stimulation (expressed in %) is determined in relation to the control sample, which constitutes appropriate materials that do not contain additives [25].

3. CONCLUSIONS

Application of packages with active substances is a good method of protecting the product from undesirable microbial growth. Thanks to them prolonging the life of many products and improving their health safety is possible. That is why proper determining the biological properties of materials is extremely important. With the application of standards and methods, many scientific-research works can be conducted in practice, which can provide an objective look at the biological properties of polymeric materials. It is well known that, regardless of the applied method or research procedure, the results should be reproducible and repetitive. Therefore, while examining the polymeric materials it is necessary to always unify and systematise their mass, thickness or surface.

It can be concluded from the above review of literature that the methods of examining the polymeric materials are still being perfected, there are gradually more and more of them and the research scope they cover is widening.

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