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BASIC BIODEGRADATION METHODS OF MATERIALS

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Abstract: Biodegradable materials and their applications are now a widespread topic of scientific activities and publications, mainly due to their ever-expanding possibilities for use in biomedical fields. Advances in the study and evaluation of materials often require different methods to determine the properties and consequences of degradation, whether changes in structure, mass changes, morphological changes or differences in the mechanical properties of the material before and after degradation. The aim of this work is to summarize the available and used methods, as well as to compare the information obtained for the design of a suitable method. The procedures are described on the basis of studies that have addressed the issue of biodegradation of materials using in vitro methods.

1 Introduction

Degradation of materials in different environments, depending on their use, is generally a frequently observed phenomenon in a wide range of disciplines, not excluding biomedical engineering. In the case of degradation assessment in a simulated human environment, it is possible to speak of a phenomenon called biodegradation.

Materials are generally prone to degradation to some extent due to various factors. In general, there are three basic categories of material degradation - physical, chemical and biological. Physical refers to the effect of force, heat, or radiation. Degradation of chemical origin refers to the destructive reactions between a material and the substances that come into contact with it. Biological degradation involves all interactions between living organisms or microorganisms and materials [1,2].

In industrial practice, degradation is a negative rather than a positive phenomenon, mainly due to changes in the structure of materials, which results in undesirable changes in the properties of materials. This can lead to reduced safety, reduced operational efficiency, but also economic problems. Degradation is therefore understood to mean processes of mostly continuous and irreversible deterioration of material properties. However, there are industries (eg ecology or bioengineering) where degradation may be desirable, in the form of biodegradable materials. Biodegradability and compostability are types of material degradation in specific environments [2].

Degradation mechanisms are closely related to chemical structures, molecular weights, the presence of microorganisms and environmental conditions. The protection of materials can be achieved to some extent by surface engineering and control of the physical, chemical and biological environment so that the surfaces of the materials are as inert as possible [3].

1.1 Mechanisms of material damage

Degradation can be initiated by external influences such as heat, humidity, chemicals, exposure to UV radiation, mold or bacteria and is promoted by mechanical stress. There are many ways in which degradation can occur. One of the most common ways is wear, for example, when material is constantly rubbing against another material. A form of degradation that affects many types of polymers is degradation caused by ultraviolet light, which affects the internal bonds of polymers. Chemical degradation is another process that can make the material less useful over time (e.g., exposing steel to hydrochloric acid).

Degradation processes often coexist in combined forms, such as corrosive wear or the occurrence of stress cracks due to the environment, where at least two degradants are involved in the degradation. While in the case of one degradant it would be a subthreshold level of damage, in combination the material fails. The most common mechanisms of damage are corrosion, wear and fatigue of the material [2,3].

1.2 Principles of biodegradation

Biodegradability of materials is nowadays a very desirable process, especially with regard to the environment, but also in the case of other fields such as. medicine, where such materials have enormous potential. Some microorganisms have a naturally occurring ability to degrade, transform, or accumulate vast amounts of compounds, including hydrocarbons, polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceuticals, radionuclides, and metals [4].

Thus, biodegradation is generally a process of decomposition of a substance by living microbial organisms. Microorganisms are one of the most adaptable biological species in the world and there is a huge amount that can contribute to degradation in different conditions. These organisms are secreted by enzymes, which break down the substance into smaller compounds by metabolic and enzymatic processes [5].

A very important factor for biodegradation is the presence or absence of oxygen. Based on this, it may be aerobic or anaerobic biodegradation [6].

The process of biodegradation can occur in different environments, which has a great influence on the course and rate of degradation. Biodegradation in marine, freshwater, soil and composting conditions is most often assessed. The behavior of materials in different conditions is different, but all environments are suitable for certain microorganisms [7].

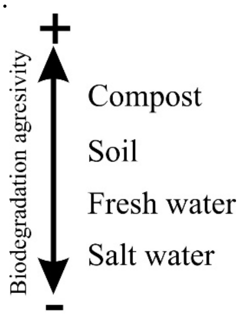

Figure 1Agresivity of biodegradation depending on environment

It is thus a systematic and complete mechanical, physical and chemical change in which the material is broken down by biotic microorganisms such as bacteria, fungi or yeast, supported by abiotic ecosystem factors such as climate and environmental conditions (i.e. acidity, humidity, temperature etc.) [3].

1.3 Biodegradation methods

Historically, biodegradation test methods were first developed for chemicals in wastewater, with the first protocols published by the OECD (Organization for Economic Cooperation and Development) in 1981. Later, biodegradation methods for other environments and also for other materials were described. In addition to the OECD, similar procedures have been published by ISO (International Standards), ASTM (American Standards), EN (European Standards), JIS (Japanese Standards) and various other national standards organizations [8,9].

In nature, biodegradation is influenced by several environmental factors and is mainly caused by enzymes produced by microorganisms, but other mechanisms may also be involved. However, during testing, conditions should be kept as constant as possible to ensure repeatability of results. It is therefore important to choose the most appropriate testing method [10].

In general, biodegradation can be assessed by two basic methods:

• *in vivo* - a method of evaluating the behavior of a biological system in its natural environment,

• *in vitro* - in experimental biology, these are methods that are performed using components isolated from their usual biological environment.

In vitro or in vivo degradation of a substance can be characterized as:

a) a primary change in the chemical structure of the substance that leads to the loss of specific properties of the substance,

(b) environmentally friendly; biodegradation to such an extent that the undesirable properties of the compound are eliminated,

c) final complete decomposition of the compound; either fully oxidized or reduced single molecules such as carbon dioxide / methane, nitrate / ammonium and water [11].

1.4 Comparison of in vitro and in vivo methods

The *in vitro* study is performed in a controlled environment, such as a test tube or petri dish. In vivo is the Latin term for "live". It refers to tests, experiments, and procedures that researchers perform on a living organism (person, laboratory animal, or plant).

While in vivo methods are usually time consuming, *in vitro* methods are fast and cost-effective methods that provide a necessary and useful complement to in vivo studies in materials testing. *In vitro* testing is a straightforward research methodology that allows more detailed analyzes and biological effects to be performed on a larger number of *in vitro* subjects than they would in animal or human experiments [12].

2 Biodegradation testing

Currently, regulations require that biodegradability claims be based on aerobic biodegradability, which usually measures oxygen consumption, CO2 production and the state of inorganic carbon intermediates. For the aerobic environment, biodegradability is measured on the basis of CO2 analysis. For an anaerobic environment, a parameter is used, which is the amount of Dissolved Organic Carbon (DOC) [13].

There are tests simulating different conditions (aquatic, terrestrial, biological conditions of the human body), based on static, semi-continuous or continuous principles, operated under aerobic or anaerobic conditions. In any of these tests, the following important factors affect biodegradation [3,11]:

• the concentration of test material, which should be high enough for the analytical methods chosen, but low enough for toxic substances or if actual environmental concentrations are to be simulated;

• physico-chemical properties of tested substances (solubility, volatility, ...)

• composition and concentration of inorganic nutrients in the test medium;

• the presence or absence of other degradable substances in the same medium for cometabolic processes;

• conditions and properties of test systems, such as volume and shape of test vessels, open or closed bottles, temperature, method of mixing or shaking, and oxygen supply;

• test duration.

In Figure 2 it can be seen the available test methods used for each degradation condition. In general, they can be divided into laboratory, simulation and field tests.

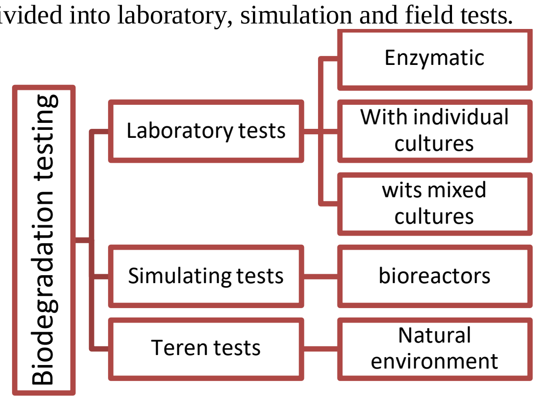

Figure 2Methodsof biodegradation testing

Laboratory tests are a suitable analytical tool for the assessment of biodegradation, while providing a synthetically generated environment with defined conditions and their results are best reproducible. Simulation tests are performed in special bioreactors that provide complex and well-defined environmental conditions. Field tests are the most appropriate choice in terms of relevance, as the material degrades directly under the natural conditions of its use, but due to the variability of environmental conditions, in most cases it is not possible to verify the results of these tests by repeated studies [14].

2.1 Factors influencing biodegradation

The basic biotic factor is the metabolic capacity of microorganisms. The rate of degradation often depends on the concentration of the substance and the number of organisms capable of metabolizing the substance [15].

Another important factor is nutrients and oxygen. Microorganisms need macronutrients and micronutrients to synthesize cellular components. In order to avoid uncontrollable microbial growth, it is necessary that sufficient nutrients and oxygen are available in a usable form and in the right proportions [3,7].

Parameters such as humidity, temperature, pH, presence resp. the absence of oxygen and the supply of the nutrients already mentioned therefore have a significant effect on the microbial degradation of the materials and must be taken into account in determining the degradation [7].

2.2 Biodegradation assessment parameters

Biodegradation is associated with several processes such as oxygen consumption, carbon dioxide formation, chemical surface change or physical losses, which are observable in the sample. There are 2 key aspects to biodegradation testing:

1. degradation parameters: weight loss, molecular weight decrease, dimensional change, deterioration of mechanical properties and analysis of surface chemistry.

2. biodegradability.

Different types of analysis methods are used for evaluation, depending on the type of parameter being assessed. Frequently used methods in the first stages of degradation are X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) or infrared spectroscopy (IRS), which are used to assess surface changes [12].

In the later stages of degradation, other parameters such as weight loss, changes in molecular weight, temperature or mechanical properties are evaluated on the basis of mass analysis. The assessment of biodegradability is determined by measuring the conversion of carbon to carbon dioxide (and methane in the case of anaerobic conditions) [16].

2.2.1 Chemical development

The basic process in aerobic biodegradation is the oxidation of carbon-based organic substances, which leads to the conversion of carbon to carbon dioxide. A certain amount of carbon may remain present in the sample as residual carbon [17].

Respirometry or the Sturm test are most often used to evaluate chemical development. In principle, these are very similar tests, with the difference that while the Sturm test accurately measures carbon dioxide production and biodegradation is determined as C → CO2 conversion, in the case of respirometric tests, O2 consumption is measured instead of CO2 production [5,14].

2.2.2 Weight

Mass loss is the most basic and widespread consequence and indicator of degradation. Weight loss measurement is a common method for detecting the biodegradation of various insoluble materials.

For example, Shrivastava et al. used weight loss as one way to detect the biodegradation of wool by two species of fungi, with weight losses of 58% and 22% for individual fungal cultures after 4 weeks of incubation.

Typically, the analysis of this property is performed by means of an experimental measurement, which consists of collecting mass data at different times (before, during and

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after the degradation process). Before each weighing, the sample must be decontaminated (using distilled or deionized water) and dried. The material can be dehumidified in three ways [18]:

- Mechanically (centrifugation, pressing, ...)
- By the action of heat (dryers)
- Physico-chemical.

2.2.3 Morphology

Biodegradation leads to a significant change in the surface structure of the material. Its process leads to continuous disruption of the sample surface, which results in the formation of cracks, microcracks and other defects, as well as changes in the dimensions of the sample [19].

Prior to analysis, the sample is usually cleaned or otherwise adjusted for microscopy. Different materials require different sample preparation procedures (grinding, polishing, etching, etc.). In the case of electron microscopy, the sample must be thoroughly dried to prevent deterioration. The samples are dehydrated by immersion in acetone. The sample is then poured into synthetic resin, cured by polymerization and post-treated (trimming, grinding) [20].

Table 1Mechanical properties and methods of testing

Mechanical property	Method of testing
Elasticity / plasticity	Compression, tensile tests, twist and bend
Stiffness, static load behavior	
Creep of the material	Creep test
Hardness	Brinell, Rockwell, Vickers exams
Toughness	Impact tests
Fatigue behavior	Wöhler fatigue test

2.2.4 Mechanical properties

One of the other consequences of biodegradation is a reduction in mechanical properties due to the disruption of the crystalline structures of the material. Mechanical properties belong to the quantitative parameters of degradation assessment, and the basis is the observation and assessment of the behavior of the material under external mechanical stress. Standardized tests are used for testing, which ensure uniform procedures for mutual comparison of material properties [20,21].

The tests cover a wide range of materials, using common test methods such as bending tests, tensile and compression tests, wear tests, fatigue tests, etc. The choice of method depends on the property of the sample under consideration.

2.2.5 Surface chemistry

The final design of materials depends, among other properties, to a large extent on their surface microstructure and behavior. In this respect, surfaces play an important role in many technological processes such as catalysis, corrosion and adhesions, these processes depending on the chemical composition of the interface [18,22].

The study of surfaces and surface phenomena at the atomic or molecular level is therefore an essential scientific field. Surface chemical analysis is a term covering a range of analytical techniques used to determine the elements and molecules present in the outer layers of solid samples.

The most commonly used techniques are Fourier Transform Infrared (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS), secondary ion mass spectrometry (SIMS) and contact angle measurement [23].

3 Design of *in vitro* degradation methods for materials

Given the similarity of the methodological procedures included in the study review, this part of the work is rather a summary of the possibilities within the methods that can be used in the assessment of *in vitro* degradation of materials. Some procedures such as corrosion ratings are specific only for metallic materials. In Figure 3 we can see a schematic of the methodological procedure that followed all the studies presented in this work.

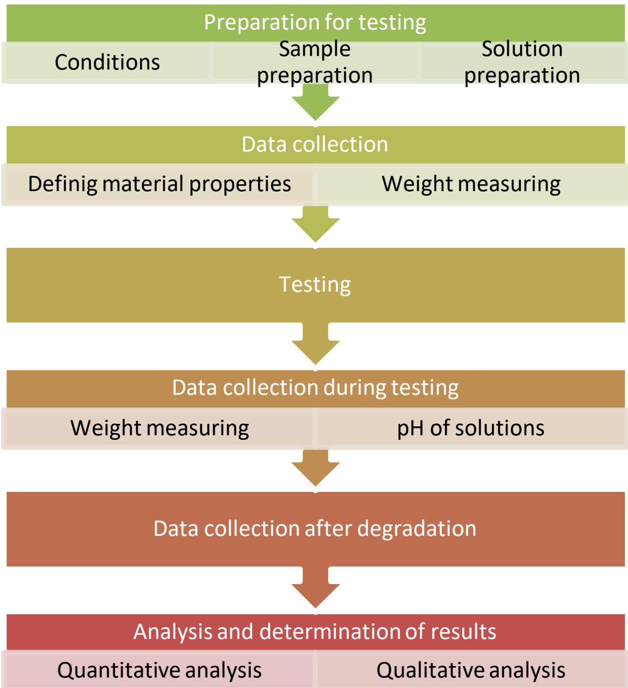

Figure 3process of testing

3.1 Choice of test method

In all the studies described in this work, agreement was found in the choice of test method. The research was based on testing by immersing samples in solution, ie on the so-called immersion test, which is a suitable method for determining biodegradation in simulated human fluid. It is also one of the most widely used methods for assessing metal corrosion.

The principle of the test is to place (immerse) the sample in a suitably selected medium for a fixed period of time, which may be interrupted in order to obtain the necessary data (eg weighing or changing the solution). Samples are weighed before, during and after testing. During testing, it is necessary to monitor and correct the pH value and also maintain the selected temperature, within the deviation.

According to the ASTM Manual for Laboratory Submersible Corrosion Testing of Metals, a versatile device should be used, most often consisting of a suitably sized flask (500-5000 ml), a reflux condenser with an atmospheric seal, an atomizing or aerating atomizer, a thermometer vessel and a temperature control device, heating equipment and sample support system. However, the equipment selected in this way is explicitly characterized for corrosion assessment.

3.2 Test conditions

The basic parameters that need to be determined in the biodegradation test are the temperature, the degradation medium with a suitable pH, as well as the duration of the test. Testing conditions vary for different materials and their uses and may vary from research to research. However, in order to obtain relevant results, it is important to maintain the input conditions throughout the process within tolerable deviations.

3.2.1 Test duration

The total duration of testing is very different compared to the studies, ranging from a few days, weeks to months. Some studies may take a year or more. The testing time chosen may depend on the potential use of the material. E.g. Bone defects usually heal much longer, so degradation behaviour should be monitored over a much longer period of time.

3.2.2 Temperature and pH value

In general, the test solution and in particular the pH have a significant effect on the outcome of the degradation experiments. A key issue for biodegradation is the simulation of physiological conditions occurring in living organisms. For this reason, the most frequently chosen pH is 7.4 and the temperature is 37 ° C (± 1 ° C), which was uniformly chosen in each of the studies. Thus, it can be argued that the most suitable values for biodegradation testing, independent of the material tested, are pH 7.4 and a temperature of 37 ° C.

3.2.3 Degradation medium

The required pH can be achieved with several solutions. Graph 3 shows that the most commonly used medium is the PBS solution used in a total of four experiments. The second is SBF, but it was used in only two studies (Zn alloy and glass-ceramics). In addition to these solutions, however, it is possible to use e.g. also Hanks' solution (HBSS), which has been used in other studies (but these are not specified). According to an overview of solutions used in the degradation of metallic materials by Di Mei et. Al. the use of complex saline solutions as a degradation medium for the Mg corrosion test for biomedical use is not completely reliable. Although PBS solutions provide a more complicated test environment such as NaCl solution, they lack several important inorganic ions (eg Ca 2+, carbonates or phosphates), which have a significant effect on Mg corrosion.

3.2.4 Data collecting

Data collection took place a total of three times during each test in each of the studies, before, during, and after degradation. The first measurement was performed on the initial characteristics of the material in terms of weight, mechanical properties and surface evaluation. The last measurement (i.e. after degradation) is in principle identical to the first measurement and is necessary to compare the results obtained. Data measurement during the test was focused only on data collection and pH control of the degradation medium.

Weighing is an integral part of degradation assessment and is one of the main parameters of the degree of degradation. It takes place throughout the process at various time intervals. However, weighing should be preceded by drying of the samples due to the absorption of the samples by the solution during the immersion time. However, not all studies report the drying process as part of the procedure.

Various methods were used to analyse the data obtained in the studies. The most commonly used (in all studies) was the SEM method, for surface evaluation. FTIR analysis is also relatively numerous, it was omitted only in the studies of metallic materials, while in them the analysis used by optical microscopy was the only one used. In addition to the conventional methods mentioned, other methods such as BEI, SEC or XRD were used in the studies.

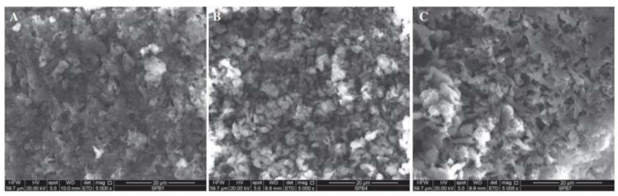

Figure 4 SEM analysis of polymer surface after 1, 4 and 7 weeks of in vitro degradation [18]

4 Conclusions

Today, there are a relatively large number of different methods for evaluating the effects of biodegradation processes affecting a material. These are most often in vivo or *in vitro* methods, which provide a wealth of information about the behaviour of the material directly in a living organism or in a solution of simulated human fluid. However, the procedures and methods chosen for biodegradation testing often differ, which can be confusing when selecting test methods for research in this area.

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References

[1] MICHEĽ, J.B.: *Náuka o materiáli*, 1st ed. Košice: TU, 2008. (Original in Slovak)

[2] REVIE, R.W.: *Uhlig's Corrosion Handbook*, Hoboken, NJ, USA: John Wiley & Sons, Inc., 2011.

[3] ALVAREZ, P.J.J., ILLMAN, V.A.: *Biodegradation Principles*, in Bioremediation and Natural Attenuation, Hoboken, NJ, USA, John Wiley & Sons, Inc., pp. 49-114, 2005.

[4] multiphysics CYCLOPEDIA, Material Fatigue, [Online], Available: https://www.comsol.com/multiphysics/material-fatigue [25 Apr 2022], 2016.

[5] FALKIEWICZ-DULIK, M., JANDA, K., WYPYCH, G.: *Handbook of Material Biodegradation, Biodeterioration, and Biostablization*, 2nd ed, Elsevier, 2015.

[6] TAHRI, N., BAHAFID, W., SAYEL, H., EL GHACHTOULI, N.: *Biodegradation: Involved Microorganisms and Genetically Engineered Microorganisms*, in Biodegradation - Life of Science, InTech, 2013.

[7] RUGGERO, F., GORI, R., LUBELLO, C.: Methodologies to assess biodegradation of bioplastics during aerobic composting and anaerobic digestion: A review, *Waste Management & Research*, Vol. 37, No. 10, pp. 959-975, 2019.

[8] HAAVE, M., HENRIKSEN, T.: Sources and Fate of Microplastics in Urban Systems, in *Handbook of Microplastics in the Environment*, T. Rocha-Santos, M. F. Costa, and C. Mouneyrac, Eds. Cham: Springer International Publishing, pp. 849-875, 2022.

[9] KRAUKLIS, A.E., KARL, Ch.W., ROCHA, I.B.C.M., BURLAKOVS, J., OZOLA-DAVIDANE, R., GAGANI, A.I., STARKOVA, O.: Modelling of Environmental Ageing of Polymers and Polymer Composites—Modular and Multiscale Methods, *Polymers*, Vol. 14, No. 1, pp. 1-44, 2022.

[10] RYAN, C.A., BILLINGTON, S.L., CRIDDLE, C.S.: Methodology to assess end-of-life anaerobic biodegradation kinetics and methane production potential for composite materials, *Composites Part A: Applied Science and Manufacturing*, Vol. 95, pp. 388-399, 2017.

[11] BOWMER, T., LEOPOLD, A., SCHAEFER, E., HANSTVEIT, R.: Strategies for selecting biodegradation simulation tests and their interpretation in persistence evaluation and risk assessment, in *Simulation Testing of Environmental Persistence (STEP)*, pp. 1-62, 2004.

[12] DE WILDE, B.L.: *Biodegradation Testing Protocols*, ACS Symposium Series, Vol. 1114, pp. 33-43, 2012.

[13] VAVERKOVÁ, M., KOTOVICOVÁ, J., ADAMCOVÁ, D.: Testing the biodegradability and biodegradation rates of degradable/biodegradable plastics within simulated environment, *Infrastructure and Ecology of Rural Areas*, Vol. 12, pp. 93-101, 2011.

[14] KOLKA, R., WEISHAMPEL, P., FRÖBERG, M.: *Measurement and Importance of Dissolved Organic Carbon*, in Field Measurements for Forest Carbon Monitoring, Dordrecht: Springer Netherlands, 2008.

[15] TOSIN, M., WEBER, M., SIOTTO, M., LOTT, C., DEGLI INNOCENTI, F.: Laboratory Test Methods to Determine the Degradation of Plastics in Marine Environmental Conditions, *Frontiers in Microbiology*, Vol. 3, pp. 1-9, 2012.

[16] AL-SALEM, S.M., SULTAN, H.H., KARAM, H.J., AL-DHAFEERI, A.T.: Determination of biodegradation rate of commercial oxo-biodegradable polyethylene film products using ASTM D 5988, *Journal of Polymer Research*, Vol. 26, No. 7, pp. 1-7, 2019.

[17] CHEN, Y.C., HSU, P.Y., TUAN, W.H., CHEN, C.Y., WU, C.J., LAI, P.L.: Long-term in vitro degradation and in vivo evaluation of resorbable bioceramics, *Journal of Materials Science: Materials in Medicine*, Vol. 32, No. 1, pp. 1-11, 2021.

[18] CASARIN, S.A., MALMONGE, S.M., KOBAYASHI, M., AGNELLI, J.A.M.: Study on In-Vitro Degradation of Bioabsorbable Polymers Poly (hydroxybutyrate-co-valerate) - (PHBV) and Poly (caprolactone) - (PCL), *Journal of Biomaterials and Nanobiotechnology*, Vol. 02, No. 03, pp. 207-215, 2011.

[19] ARYAL, S.: Gel Permeation Chromatography- Definition, Principle, Parts, Steps, Uses, *Microbe Notes*, [Online], Available: https://microbenotes.com/gel-permeation-chromatography [25 Apr 2022], 2022.

[20] SCHARNWEBER, D.: *Biodegradation of Metals*, in Encyclopedia of Materials: Science and Technology, Elsevier, 2001.

[21] ZHENG, Y.F., GU, X.N., WITTE, F.: Biodegradable metals, *Materials Science and Engineering: R: Reports*, Vol. 77, No. March, pp. 1-34, 2014.

[22] MEENAMBIGAI, P., VIJAYARAGHAVAN, R., GOWRI, R.S., RAJARAJESWARI, P., PRABHAVATHI, P.: Biodegradation of Heavy Metals – A Review, *International Journal of Current Microbiology and Applied Sciences*, Vol. 5, No. 4, pp. 375-383, 2016.

[23] BRIASSOULIS, D., MISTRIOTIS, A., DE WILDE, B., NOORD, D.: *Standard testing methods & specifications for biodegrada- tion of bio-based materials in soil - a comparative analysis*, International Conference of Agricultural Engineering, Zurich, 2014.

Review process

Single-blind peer review process.