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Hydrophilic-hydrophobic rubber composites with increased susceptibility to biodegradation

Summary — The vulcanizates of epoxidized natural rubber of epoxidation degree equal 50 mol % (ENR50) were prepared. The samples were cured using two methods (traditional or unconventional one) and contained different amounts of keratin hydrolyzate as biologically active additive. Mechanical properties of these vulcanizates and their susceptibility to biological degradation by mildew fungi attack were investigated. The results of biodegradation of selected vulcanizates done either without nutrition (method A) or with it (method B) were presented. Additionally the soil test of biodegradation of the composites was done. The use of keratin hydrolyzate in rubber blends increased the susceptibility of the vulcanizates to biodegradation.
Key words: epoxidized natural rubber, biodegradation, composites, keratin hydrolyzate, mildew fungi.

HYDROFILOWO-HYDROFOBOWE KOMPOZYTY GUMOWE O ZWIĘKSZONEJ PODATNOŚCI NA BIODEGRADACJĘ

Streszczenie — Przygotowano wulkanizaty epoksydowego kauczuku naturalnego o stopniu epoksydacji 50 mol % (ENR50) usieciowanego dwiema metodami (tradycyjnie oraz w sposób niekonwencjonalny) i zawierające różne ilości biologicznie aktywnego hydrolizatu keratyny. Zbadano właściwości mechaniczne (rys. 1—3, tabela 3), a także podatność wspomnianych wulkanizatów na degradację biologiczną za pomocą grzybów pleśniowych. Przedstawiono wyniki biodegradacji wybranych wulkanizatów prowadzonych bez (metoda A) lub z użyciem (metoda B) pożywki (tabele 4 i 5). Dodatkowo wykonano testy biodegradacji glebowej tych kompozytów (tabela 6). Wyniki te wskazują, że zastosowanie w mieszankach kauczukowych hydrolizatu keratyny zwiększyło podatność otrzymywanych wulkanizatów na degradację biologiczną.
Słowa kluczowe: epoksydowany kauczuk naturalny, biodegradacja, kompozyty, hydrolizat keratyny, grzyby pleśniowe.

The growing number of publications on biodegradability and biodegradable materials indicates persistent interest in and demand for this type of materials. Hence, it seems advisable to continue the studies aimed at the preparation of biodegradable composites. Among various possible processes of waste disposal, including polymeric waste, biodegradation or biorecycling is now perceived as one of the most attractive solutions [1]. It is a more and more widely accepted opinion that the production of polymers that would be degraded in the environment can help to solve the problem of growing amounts of waste [2]. Currently there are many methods of the assessment of polymer biodegradability, including soil test (composting) [3], modified Sturm's method [4] and enzymatic degradation [5]. These tests allow to assess properly the biodegradability of tested polymers.

At present, in the world market there are available more and more goods which contain in their compositions natural polymers such as starch of various origin that is the most frequently used ingredient. The presence of a hydrophilic polysaccharide in a polymer exposed to the effects of the environment causes deterioration of its original properties and a composition is easier destructed under the influence of external conditions [6]. In our opinion, it is also possible to use various protein hydrolyzates. The aim of this study was to prepare the composities with keratin hydrolyzate used earlier mainly in the cosmetic industry (hair and nail nutrients, bath fluids, etc.). This is a soluble protein produced by controlled hydrolysis of sheep's or goat's wool. An additional advantage of this biologically active substance, similarly to all other natural polymers, is its better acceptance by biologically active compounds (biodegradability: 99%) in comparison with most synthetic materials, easy availability in large amounts and complete non-toxicity [7].

Considering the growing use of polymeric materials and their microbiological resistance [8], our attention was focused on the preparation of practically useful

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composites that would be degraded in the environment owing to the incorporation of biopolymers into the elastomeric system.

EXPERIMENTAL

Materials

Chemical materials

One grade of rubber was studied: epoxidized natural rubber showing 50 mol % degree of epoxidation (ENR50) manufactured by Kumpulan Guthrie Berhad, Malaysia. This elastomer was crosslinked using conventional system *i.e.* sulphur and the accelerator which was *N*-cyclohexyl-2-benzothiazolylsulphenamide (CBS) or unconventional system *i.e. cis*-1,2,3,6-tetrahydrophthalate anhydride and 3-dimethylaminophenol as catalyst. The keratin hydrolyzate manufactured by Proteina Natural Protein Plant S.C. Poland was used as a biologically active substance.

Formulation of composites [given in parts per hundred of ENR50 (phr)] where following: keratin hydrolyzate — 0—40; lecithin — 0—0.8; sulphur — 1.5 or *cis*-1,2,3,6-tetrahydrophthalate anhydride — 5; zinc oxide — 5; stearic acid — 2; calcium stearate — 3; CBS — 1.5 or 3-dimethylaminophenol — 0.5; magnesium oxide — 5; calcium oxide — 2.

Biological material

Hyphal fungi are the most effective organisms in the biodegradation of plastics and therefore they were used as test organisms. The selection of mildew fungi was consistent with Standard PN-EN ISO 846 (Dec. 2002 "Plastics. Assessment of the effects of micro-organisms"). The following mildew fungi were used in the experiments: *Aspergillus niger*, *Penicillium ochrochloron*, *Aspergillus terreus*, *Scopularopsis brevicaulis*, *Aureobosidium pullulans*, *Trichoderma viride*, *Paecilomyces variotti* and *Chaetomium globosum*. The strains were derived from the Collection of Pure Industrial Microbe Cultures of the Institute of Fermentation Technology and Microbiology (Technical University of Łódź). The mildew fungi strains were stored in a 5°Blg sweet wort.

Nutrient media

The low-value nutrient medium consisted of: $NaNO_3$ 2.0 g, KH_2PO_4 0.7 g, K_2HPO_4 0.3 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.01 g, agar 20 g, water 1000 ml, pH was 6.0—6.5.

The nutrient medium of full value had the same composition as above plus 30 g of glucose.

Methods

Vulcanization

Rubber compounds were mixed on a two-roll mill at the rolls temperature of 27—37 °C. The time of vulcanization kinetics was measured in accordance to PN-ISO 3417 using an oscillating disc vulcameter at temperature of 160 °C. The vulcanization of rubber blends was carried out at the same temperature and time determined in vulcameter in steel molds placed between electrically heated plates of a hydraulic press. The mechanical properties of vulcanizates were determined according to PN-ISO 37:1998 by means of a tensile testing machine "ZWICK", model 1435, linked to a computer with a proper software.

The resistance to thermal ageing was carried out (PN-ISO 188:2000) in a thermostated drier with hot air circulation at temperature of 70 °C for 7 days. After this time, samples were appropriately tested.

The resistance to ageing was calculated from the following relationship:

$$K = [TS' \cdot EB']/[TS \cdot EB]$$

where: *K — coefficient of ageing resistance, TS' — tensile strength after ageing, TS — tensile strength before ageing, EB' — elongation at break after ageing, EB — elongation at break before ageing*.

Biodegradation of vulcanizates

Tests were carried out according to Standard PN-EN ISO 846.

Two test methods were used to evaluate the effects of mildew fungi on the material under investigation. The method A is used to assess the natural resistance of the material in the absence of any nutrient. The method B is used in the case of expected surface contamination to check the fungistatic properties of the tested material and the effect of surface contamination of the material on its resistance.

Preparation of biological material

Suspensions of micro-organisms with a density of 10^6 spores/ml in physiological salt solutions were prepared. The mixtures of spores obtained by mixing of the equal volumes of suspensions of single strains were used in the tests.

Testing of natural resistance of materials (method A)

The tested material, previously disinfected with 70 % ethanol was placed into the low-value medium (see: Nutrient media), then the suspensions of microorganisms were applied uniformly to the surfaces of media and samples followed by their incubation at temperature of 29 °C for 4 weeks. After this period, the growth of microorganisms on the surfaces was evaluated.

Assessment of the fungistatic effect (method B)

Samples of the tested material were placed into the medium of full value (see: Nutrient media), then the suspensions of microorganisms were applied uniformly followed by incubation at temperature of 29 °C for 4 weeks. After this period, the growth of microorganisms on the surfaces of media and samples were evaluated and the zones of growth on the samples were observed.

Testing of rubber biodegradation in soil (Soil tests)

Samples were placed in an active compost soil and incubated at temperature of 30 °C and 80 % of relative

humidity. After 50 days of incubation the appearances of the samples were evaluated using optical microscopy.

Interpretation of the results (visual evaluation of fungi growth)

The samples exposed to biodegradation according to the methods A and B were evaluated by means of a standard microscope and a stereoscopic microscope (magnification 60 times), using an assessment scale in accordance with PN-EN ISO 846 (Table 1).

T a b l e 1. Evaluation of the growth of microorganisms according to PN-EN ISO 846

Growth intensity	Rating
No visible growth under the microscope	0
A growth invisible to the naked eye, visible under the microscope	1
A growth visible to the naked eye, covering up to 25 % of sample surface	2
A growth visible to the naked eye, covering up to 50 % of sample surface	3
A considerable growth, covering more than 50 % of sample surface	4
An intensive growth covering the total sample surface	5

T a b l e 2. Evaluation of the test materials according to PN-EN ISO 846

Method	Growth intensity	Evaluation of the test material
A	0	the material is not a nutrient for microorganisms
	1	the material contains substances that constitute a nutrient for microorganisms or it is contaminated to a small extent that causes a slight growth
	from 2 to 5	the material is not resistant to the action of microorganisms and contains substances being nutrients for their growth
B	0	a strong fungistatic effect
	0 + inhibition zone	a strong fungistatic effect comprising the zone around the sample
	from 2 to 5	no fungistatic effect

The interpretation of the test results was made according to PN-EN ISO 846 (Table 2).

RESULTS AND DISCUSSION

Based on our previous studies, from among several series of rubber compounds with various keratin hydrolyzate contents (from 5 to 40 phr), the samples showing the best mechanical properties were selected for biodegradation. It was observed that the incorporation of 5 phr of hydrolyzed protein into the elastomer composition improved some basic mechanical properties such as tensile strength (Fig. 1). However, the rubber filled with the hydrolyzate showed decreased elongation at break in comparison to the vulcanizate without the protein filler (Fig. 2).

The effect of elevated temperature on the behavior of samples containing keratin hydrolyzate was also examined to assess the rubber durability. Based on the obtained results, it turned out that the incorporation of this

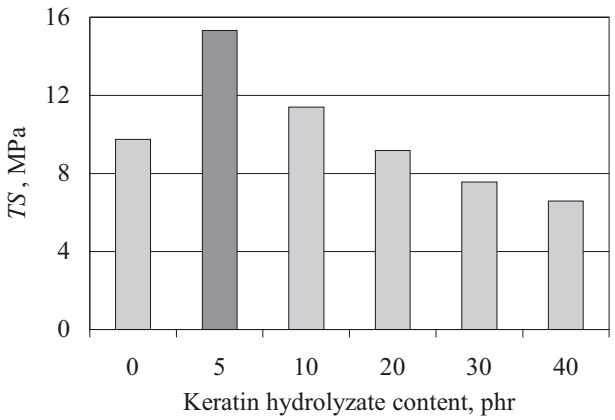

Fig. 1. Influence of keratin hydrolyzate content on tensile strength (TS) of ENR50 sulphur vulcanizates

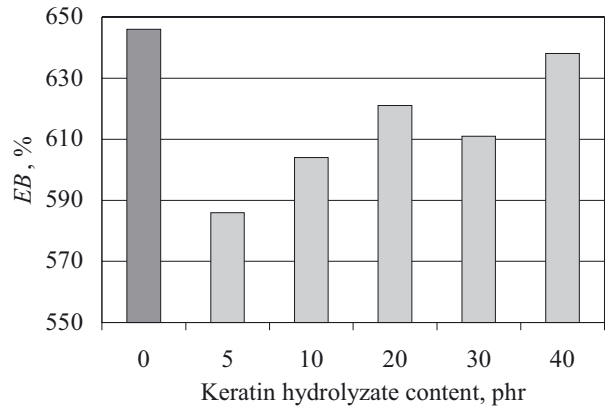

Fig. 2. Influence of keratin hydrolyzate content on elongation at break (EB) of ENR50 sulphur vulcanizates

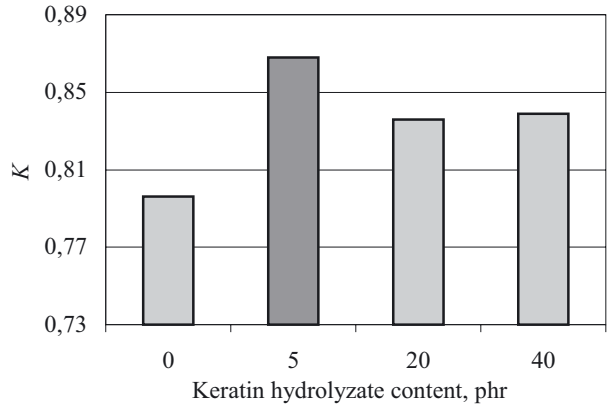

Fig. 3. Influence of keratin hydrolyzate content on the coefficient of ageing resistance (K) of ENR50 sulfur vulcanization

protein into the tested rubber increased the coefficient of ageing resistance (*K*) (Fig. 3).

Vulcanizates with unconventional crosslinking agents containing the protein hydrolyzate were subjected to the same analysis. It turned out that the hydrolyzate showed no negative effect on the mechanical properties of vulcanizates at the level of 5 phr (Table 3) similarly as in the case, discussed above, of the sulfur vulcanizates.

During the next stages of tests the progress of biological degradation of the obtained composites was assessed, taking into consideration the processes occurring in the environment as well as those taking place only under the influence of selected microorganisms.

T a b l e 3. Mechanical properties of ENR50 cross-linked with anhydride containing the keratin hydrolyzate[*)]

Keratin hydrolyzate, phr	$\nu_T \cdot 10^{-5}$ mol/dm^3	*M100* MPa	*TS* MPa	*EB* %
0	12.69	0.70	5.55	482
5	10.46	0.88	5.58	464
10	7.55	0.53	4.32	562

*) ν_T — crosslinking density of vulcanizates was calculated from the measurements of equilibrium swelling in toluene; *M100* — modulus at 100 % elongation; *TS* — tensile strength; *EB* — elongation at break.

T a b l e 4. Visual assessment of microorganisms' growth on the sample surface as a carbon source (Method A); denotations of samples described in the text

Microorganism	Sample		
	I	II	III
fungi	0	0	3
natural microflora	1	3	4

In the tests with no additional source of carbon from the culture medium (method A), no growth of mildew fungi on the sample crosslinked by conventional system in the absence (sample I) or presence (sample II) of keratin hydrolyzate was observed. In the case of the sample cured by unconventional system, with 5 phr of the hydrolyzated protein (sample III), the intensity of fungi growth was assessed as 3, what means that 50 % of the sample surface was covered by the growth of mildew micro-flora (Table 4). The natural micro-flora that was on the surface of test material (derived from the raw materials, air, equipment surface, etc.) showed the growth onto all vulcanizates, although with different intensities; the weakest growth was on the sample I and the most intensive one on the sample III. The observed very weak fungi growth on the surface of sulphur vulcanizate (sample I) is probably due to the pollution of the test material with an organic matter (dust), while in the samples II and III, the nutrient substance for microorganisms consisted of the keratin hydrolyzate.

In the presence of carbon source (method B) on the surface of tested samples, the growth of the mixture of mildew fungi and natural micro-flora was more intense than in the method A. Its intensity was assessed from 1 to 5, which indicates no fungistatic effect, thus surface pollution of the test material with easily bio-available components increased its susceptibility to the attack of mildew fungi (Table 5). The most intensive fungi growth covering the whole sample surface was observed in the case of vulcanizate denoted as sample III. Moreover, the surface became worse.

T a b l e 5. Visual assessment of microorganisms' growth on the sample surface in the presence of a carbon source (Method B); denotations of samples as in the table 4

Microorganism	Sample		
	I	II	III
fungi	1	2	5
natural microflora	2	3	5

After 50 days of incubation of the tested samples in soil, the changes on the sample surfaces were assessed by microscopy. The results are given in Table 6. In each case the macroscopic changes such as surface dulling were observed. Moreover, on the samples I and III discolorations were found indicating the soil micro-flora activity that resulted in the formation of pigments or

T a b l e 6. The results of soil tests; denotation of samples as in table 4

Sample	Morphological changes	The appearance of samples	
		control	after 50 days incubation
I	surface fogging, the presence of gray, brown and pink spots		
II	surface fogging		
III	surface fogging, discoloration the presence of roughness and pits, dark spots and light-gray blooms on the surface, characteristic earth-like smell		

reactions between microorganisms and the test material. The vulcanizate most susceptible to the action of microorganisms was sample III. Its surface showed pits and structural changes on the surface as well as colorations and blooms of microorganisms (Table 6). These observations are consistent with the results obtained using methods A and B.

CONCLUSIONS

— Incorporation of 5 phr of the keratin hydrolyzate into the ENR50 compound improves tensile strength of vulcanizate. On the basis of the performed tests, it was also observed that the addition of hydrolyzed protein to rubber compounds increased vulcanizate resistance to thermal ageing.

— The tested materials are characterized by different susceptibility to biodegradation — the most susceptible to the mildew fungi attack is vulcanizate denoted as sample III which contains 5 phr of the keratin hydrolyzate and anhydride curing system. The growth of the fungi mixture was observed also when there was no other carbon source in the tested medium, what indicates that the keratin hydrolyzate was used as a nutrient substance. The growth of fungi on the remaining vulcanizates was observed only in the presence of an additional carbon source. Thus, these materials will be attacked by mildew fungi under conditions of surface pollution.

— The micro-flora present on the vulcanizate surface is more effective on tested materials than the collection of microorganisms in the artificially created mixture. This results from the fact that the organisms which are the best adapted to the existing conditions live on the surface of materials.

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