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PVP BASED MATERIALS: BIODEGRADATION IN DIFFERENT ENVIRONMENTS

ANALIZA MATERIAŁÓW NA BAZIE PVP: BIODEGRADACJA W RÓŻNYCH ŚRODOWISKACH

Abstract: The research deals with biodegradation of films prepared from polyvinylpyrrolidone and polylactic acid (PVP/PLA). Biodegradation of PVP/PLA films was supported by the following additives: 1-methyl-2-pyrrolidone, 1-octyl-2-pyrrolidone, acrylamide and N-acetyl-L-phenylalanine according to the previous study. The films were prepared by a solvent casting technique. Biodegradation was observed using the respirometric method in different environments. The films subjected to biodegradation were analyzed by scanning electron microscopy and Fourier transform infrared spectroscopy. It was found that the films are substantially degraded, but not in the biological way; PVP was quickly removed in presence of water because of its easy solubility. In contrast, this fact could support biodegradation of PLA, which becomes more available for microorganisms when PVP leaves PLA matrix.

Keywords: polyvinylpyrrolidone, polylactic acid, biodegradation, soil, activated sludge water, digested sludge

Introduction

Polyvinylpyrrolidone (PVP) was first synthesized in 1939 by Reppe. It is a white or yellow water-soluble linear polymer containing a pendant lactam ring usually occurring in the form of hygroscopic powder with significant physical and chemical properties. According to the molecular weight and viscosity several types of this polymer are distinguished [1], therefore, the usage of PVP is so various. It could be found in households, where people are in contact with it almost daily in the form of detergents, cosmetics and pharmaceutical products. This compound is also an additive to polymer blends used for production of sticky tapes, paints and for manufacture of packaging materials. Actually, it has a very low toxicity, so it can be also used in food industry as a stabilizer or a thickening agent [2].

There is apparently only one problem in this context - the resistance of PVP to biodegradation. Accumulation of this material could pose a potential threat for

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environment, because of its unclear behavior and fate in nature. Due to its physical and chemical properties it could increase the mobility of other pollutants.

Julinova et al. [3] suggested theoretical decomposition of PVP in the presence of specific types of microorganisms. This suggestion is divided in two steps. First, a lactam ring is broken under the influence of gamma-lactamases enzymes in the place of a heterocycle's amide bond to create secondary amine. In the next step, this product could be decomposed in the presence of amine oxidase enzymes into methylamine and propionic acid.

However, practical studies of PVP biodegradation, without any previous modifications of the sample, are almost in line with results indicating that PVP is largely resistant to biological degradation [3-6].

One of the options how to support biodegradation of PVP could be its decomposition in the presence of cosubstrates [5, 6]. The following substances were studied: 1-methyl-2-pyrrolidone (NMP) and 1-octyl-2-pyrrolidone (OP) - having similar structure as PVP, and acrylamide (AC) with N-acetyl-L-phenylalanine (APhA) - as substances during whose biological degradation the mentioned gamma lactamases are produced.

The process of PVP removal requires the knowledge of photo-Fenton reactions and photo-oxidative degradations. The biodegradation rate of thus modified PVP samples has not been overcome yet [7-10].

PVP is becoming a very popular component of various polymer blends with a huge potential of usage in many industries. It can be combined with substances such as starch [11], carboxymethylcellulose [12], polyvinylalcohol [10], caprolactone [13, 14], chitosan [15], polyethyleneglycol [16] or polydimethylsiloxane [17]. The study by de Paula et al. deals with polymer films prepared from PVP and polylactic acid (PLA) [18]; this material finds its application in medicine and in biotechnology. Results of this research point to considerable biodegradation of this polymer blend.

Another frequently used polymer is PLA, whose biodegradation is often mentioned with composting. Possible biodegradability of this material was first described in 1981 [19] and has been investigated in many studies, on the base of which it is considered a biodegradable polymer. Nonetheless, according to the studies of biodegradation of PLA in soil this substance is still more or less resistant. This type of environment most closely represents real conditions [20-23].

Although considerable research has been devoted to continuous improvement of properties of the polymer blends containing PVP, rather less attention has been paid to its actual biodegradation.

Thus, the aim of this paper is to study the biodegradability of PVP/PLA films based on de Paula's research [18] and the biodegradability of PVP/PLA system with addition of NMP, OP, AC and APhA [3].

Materials and methods

Materials

The following materials were used for the preparation of polymer blends: polyvinylpyrrolidone (PVP; 10 kDa), 1-methyl-2-pyrrolidone (NMP), 1-octyl-2-pyrrolidone (OP), acrylamide (AC), N-acetyl-L-phenylalanine (APhA) were purchased from Sigma Aldrich at a purity of 98%. Chloroform was produced by Penta

Company and polylactic acid (PLA) was obtained from PLA2003D polylactic acid was purchased from NatureWorks® Ingeo™ (USA).

Biological materials (inoculum)

The soil environment was simulated by clay soil and commercial garden compost which were purchased from Agro CS a.s. (Czech Republic). These types of soil were mixed in the ratio of 1:5. Thereafter, 100 cm³ of liquid medium was applied to the mixture. Thus prepared mixed soil was left before the preparation of the main experiment for 7 days to acclimatize.

The water environment was simulated by aerobic or anaerobic activated sludge, obtained from waste-water treatment plant (Zlin-Malenovice, Czech Republic), suspended in the liquid medium. The broth corresponded to the medium from soil experiment for aerobic conditions. However, the broth for anaerobic conditions was prepared slightly differently: a 1 dm³ volumetric flask was filled with 500 cm³ of distilled water. Then, 1 cm³ of each of solutions: CaCl₂ (27.5 g·dm⁻³), FeCl₃·6H₂O (0.25 g·dm⁻³), MgSO₄·7H₂O (22.5 g·dm⁻³) and of solution containing trace elements (0.75 g·dm⁻³ H₃BO₃, 0.05 g·dm⁻³ (NH₄)₆Mo₇O₂₄·4H₂O, 0.18 g·dm⁻³ CoSO₄·7H₂O, 0.5 g·dm⁻³ CuSO₄·5H₂O, 0.1 g·dm⁻³ ZnSO₄·7H₂O, 3 g·dm⁻³ FeSO₄·7H₂O) was gradually added. After it, 40 cm⁻³ of phosphate buffer (8.2 g·dm⁻³ KH₂PO₄, 21.75 g·dm⁻³ K₂HPO₄·12H₂O, 44.7 g·dm⁻³ Na₂HPO₄·12H₂O) and 50 cm⁻³ of (NH₄)₂SO₄ (10 g·dm⁻³) solution was added [3]. At the end, the solution was stirred and the volumetric flask was filled up to the mark with distilled water.

Preparation of PVP/PLA blends

The foils were prepared from PVP/PLA polymer blend by the method of casting. First, 10% (v/v) solution of PLA in chloroform was prepared by stirring for 6 h until PLA was completely dissolved; 10% (v/v) solution of PVP was prepared very similarly. In the next step, both solutions were mixed together in the ratio of 50/50. This blend was stirred for 2 hours in order to properly homogenize. In the end, the final blend was poured into the mould - Petri dishes ($d = 17.7$ cm); a thickness of films was 203 μm. Pure PVP and PLA films were also moulded from 10% (v/v) solution in chloroform. The foils were left in the air to let solvent evaporate. Finally, they were dried in a dessicator until the constant weight [18]. In this way, films of PVP/PLA were prepared in the ratio of PVP to PLA: 0/100, 50/50, 100/0. Moreover, a series of films with additives was prepared: NMP, OP, AC, APhA in the range of 1% (v/v) of the original PVP/PLA (50/50) blend. All kinds of the films were submitted to further analysis.

Fourier transform infrared spectroscopy (FTIR)

The IR spectrum analysis of the films was performed in the form of KBr pellets by the FTIR. KBr pellets were prepared from the dried films (0.1% of the KBr amount), which were dissolved in chloroform and mixed with the appropriate amount of KBr. This measurement was conducted on a FTIR-Nicolet iS10 spectrophotometer (Thermo Scientific, USA) and to evaluate the data, Omnic software (4000-500 cm⁻¹) was used.

Scanning electron microscopy (SEM)

The films were also observed by scanning electron microscopy. Previously, the samples of films were attached to the carbon tape for better conductivity (or covered with a gold layer). Then the surface of films could be studied by using the Vega II/LMU Tescan model and the images were taken at the magnification of 100× to 2 500×.

Measurement of weight loss

The weight loss of the films was measured on analytical balances Sartorius. The differences (Δw) were determined between the weight of the dried films before (w_0) and after (w_1) the biodegradation experiment - the samples were carefully cleaned and dried to the constant weight. The weight loss (%) was calculated by equation [2]:

$$\Delta w = 100 - \left(\frac{w_1}{w_0} \cdot 100 \right) \quad (1)$$

Tests of biodegradability in soil and water (aerobic, anaerobic) environment

Measuring of the biological degradation was carried out on the laboratory respirometer BI-2000 (Bioscience, Inc., USA) according to standard ISO 17556:2012 [24] in the soil environment. For this experiment two types of samples were prepared. The first were blanks containing only 500 cm⁻³ of mixed soil (endogenous respiration). The second were samples including different kinds of foils. These respirometric flasks were filled as follows: first, 250 cm⁻³ of soil was applied and then 1 g of the film was added. At the end, another 250 cm⁻³ of soil was applied. Finally, the whole equipment was prepared for fluent registration of BOD. All tests were performed in triplicate. The biodegradation in percentage (D) was calculated from equation [3]:

$$D = \frac{BOD_{spec}}{COD_{Cr}} \cdot 100 \quad (2)$$

where BOD_{spec} is measured biological oxygen demand for samples corrected for biological oxygen demand for blanks and COD_{Cr} is chemical oxygen demand.

The COD_{Cr} for all films was experimentally investigated as follows: an appropriate amount of the films (measured on a Mettler MX-5 microbalance, Toledo International Inc., USA) was transferred to the test tubes filled up with 2 cm⁻³ of distilled water and digested for 2 hours at 150°C according to ISO 15705:2002 [25] by the Micro Digestion Method.

The sample biodegradation was also observed in an aqueous aerobic medium according to standard CSN EN ISO 9408 [26] in a closed respirometer Micro-Oxymax (Columbus Instruments, USA). The samples (10 mg) were placed into the reaction flasks, after that 45 cm⁻³ of biomedium and 5 cm⁻³ of the inoculum of aerobic activated sludge were pipetted. The actual concentration of biomass was set to 500 mg·dm⁻³ in this experiment. Thus prepared flasks were placed in the appropriate position of respirometer and sealed. The level of pH was maintained during the test within a 7.0 ± 0.5 interval. Samples of the suspension for determining the dissolved organic carbon, pH and dry matter of biomass were always withdrawn at the start and end of the test. Endogenous respiration was investigated concurrently. All tests were performed in triplicate. The rate of biodegradation in percentage (D_{CO_2}) was calculated by equation [26]:

$$D_{CO_2} = \frac{w_{c-CO_2}}{w_{theor.c-film}} \cdot 100 \quad (3)$$

where w_{c-CO_2} is production of carbon [mg] in the form of CO_2 , and $w_{theor.c-film}$ stands for the amount of carbon in the sample [mg].

The biodegradation was determined by the bottle test in anaerobic conditions corresponding to standard CSN EN ISO 11734 [27]. The microbial gas production in these bottles was periodically recorded on the gas chromatograph GC Agilent 7890 A and the detected signals were evaluated by Clarity software. This experiment was realized in 500 cm³ flasks with 100 mg of PVP/PLA and PVP/PLA/(NMP, OP, AC, APhA) films or with standard. Finally, 100 cm³ of anaerobic sludge inoculum was applied into the flasks. The experiment was set to support the mezophilic microbial cultures, which require the temperature around 38°C. All tests were performed in triplicate. The anaerobic biodegradation in percentage (D_g) was evaluated according to equation [27]:

$$D_g = \frac{(w_{C(CO_2)} + w_{C(CH_4)})_s - (w_{C(CO_2)} + w_{C(CH_4)})_{BL}}{w_c T_c} \cdot 100 \quad (4)$$

where $w_{C(CO_2)}$ and $w_{C(CH_4)}$ are the productions [mg] of carbon in the form of carbon dioxide and methane in samples (S) and in blank (BL, endogenous production) during biodegradation of films which contained T_c [%] of carbon. The total production [mg] of carbon is identified as w_c .

Test of water solubility

The solubility of the films was analyzed in a supplementary test explaining the behavior of polymer films. First, the defined amount of distilled water was applied into Erlenmeyer's flasks, then the samples of films were added in them. These samples were dried and weighed on analytical balances before the main experiment. The dissolved amount of films was observed by taking samples in selected intervals. The samples were then analyzed on an automated carbon analyzer - Shimadzu TOC 5000 A. The test was running until equilibrium was reached. Water solubility in percentage (R) was evaluated to equation (5):

$$R = \frac{c_w}{c_s} \cdot 100 \quad (5)$$

where c_w is actual amount of carbon in water, c_s is the total carbon contained in films.

Results and discussion

Biodegradation of the films obtained by the cast technique was tested in different environments. Figure 1 shows results from all types of performed biodegradation tests. As can be seen, degradation reaches only 1% for the pure PVP film and 4% for the pure PLA film in the soil environment. However, the PLA biodegradation is higher (around 19%) in the water environment under aerobic conditions. Thus, it can be assumed that a higher rate of hydrolysis of PLA occurs in this environment. Nonetheless, for technical reasons, the biodegradability of these pure polymer films was not tested in anaerobic conditions.

The biodegradation rate of the PVP/PLA film was recorded as 4% in the soil environment. The results obtained from the test in water anaerobic conditions are quite similar in the terms of biodegradation experiments. Based on this information it cannot be specified whether PLA or PVP is mainly responsible for the measured degradation. To determine it, a series of more specific tests would have to be carried out.

A very interesting rate of biodegradation was found during the decomposition of films containing additives. The highest rate of biodegradation was measured for the PVP/PLA/NMP film and the lowest for PVP/PLA/AC. The biodegradation of polymer blends with additives except for NMP was not tested in the water environment under aerobic conditions.

The recorded percentage of biodegradation cannot be considered significant for conceivable degradation of contaminants present in PVP, which could have remained there from the process of its production. This pattern may be caused by the sorption of PVP to soil particles. We also cannot exclude possible formation of complexes with PVP and soil components or with microbial enzymes which could lead to the recorded inhibition. The possibility of the primary decomposition of PVP that could lead to the formation of metabolites inhibiting soil microorganisms cannot be omitted. It is obvious that the measured biodegradation of films containing additives corresponds with the biodegradation of the additive itself.

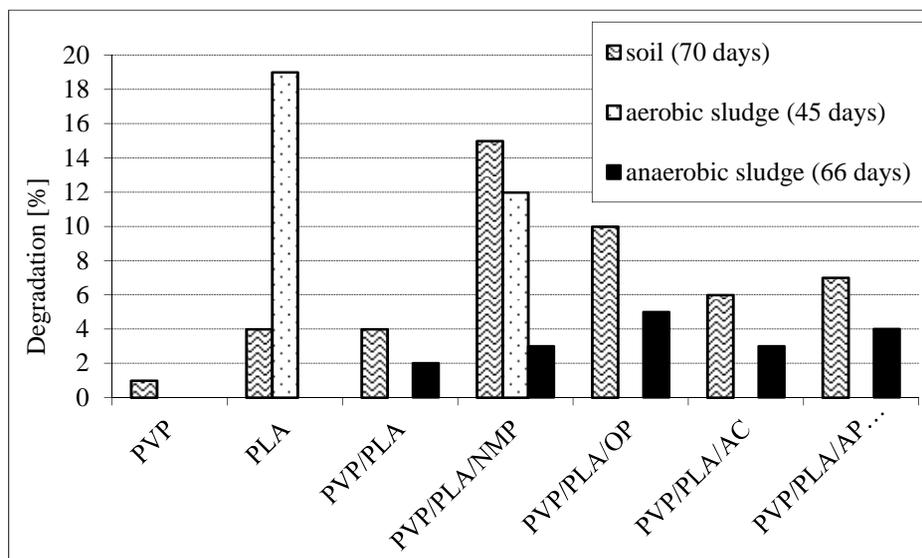


Fig. 1. Biodegradation of films in different environments

The weight loss of films was also investigated before and after all biodegradation experiments by using the gravimetric method. Figure 2 presents the results of these tests. It was found that the decrease in every polymer blend film is about 45%. The weight loss of the films with additives is demonstrably higher than that of actual PVP/PLA. The content of easily soluble additive, except for OP, may be a major cause of a higher rate of the

decrease. Probably, due to the difficult solubility of OP in water, a lower decline can be observed in its case.

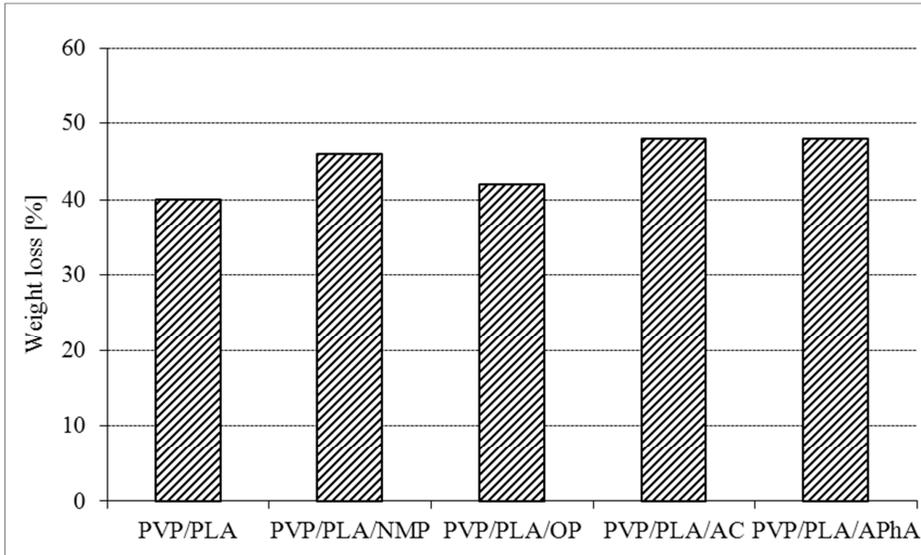


Fig. 2. Weight loss of films after biodegradation tests in soil environment (70 days)

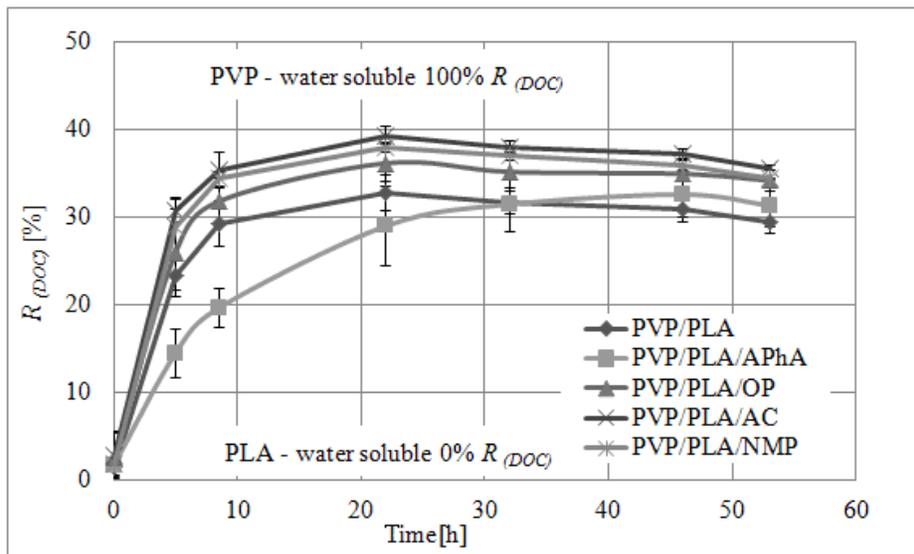


Fig. 3. Water solubility of PVP/PLA (50/50) films without and with additives until equilibrium was reached (53 h), \pm standard deviation ($n = 3$)

Figure 3 demonstrates that this fact corresponds with the results of the solubility measurement. As shown here, the solubility of the films was approximately the same. However, a few divergences are observed between the results from the weight loss and the water solubility test. It can be seen that the values differ by about 10%. This fact may be caused by the inhomogeneity of the particular films. Nevertheless, the trend of these tests is quite similar except for PVP/PLA/APhA film, whose loss is lower in the solubility test. The cause may be the inhomogeneity of the system again. The figure also shows very good water solubility of pure PVP film (100%) and insolubility of PLA (0%).

The value of about 45% weight loss of films practically confirmed that PVP was removed from the matrix PVP/PLA (50/50). Nonetheless, it was appropriate to analyze IR spectrum to prove this claim.

Figure 4 shows some changes in the infrared (IR) spectrum of the PVP/PLA films. This spectrum was obtained by Fourier transform infrared spectroscopy and shows two different courses - before and after biodegradation. As can be seen, a several changes are registered in the IR-spectrum after the biodegradation experiments, particularly in the range of wavenumber specific for PVP. For instance, the peak observable for a -OH group in presence of PVP decreases at around 3500 cm^{-1} . Other declines are registered in the peaks corresponding to the C=O group from the lactam part of PVP at 1666 cm^{-1} and to a C-N bond at 1286 cm^{-1} . This decline was highly likely caused by dissolution of PVP from the test specimens.

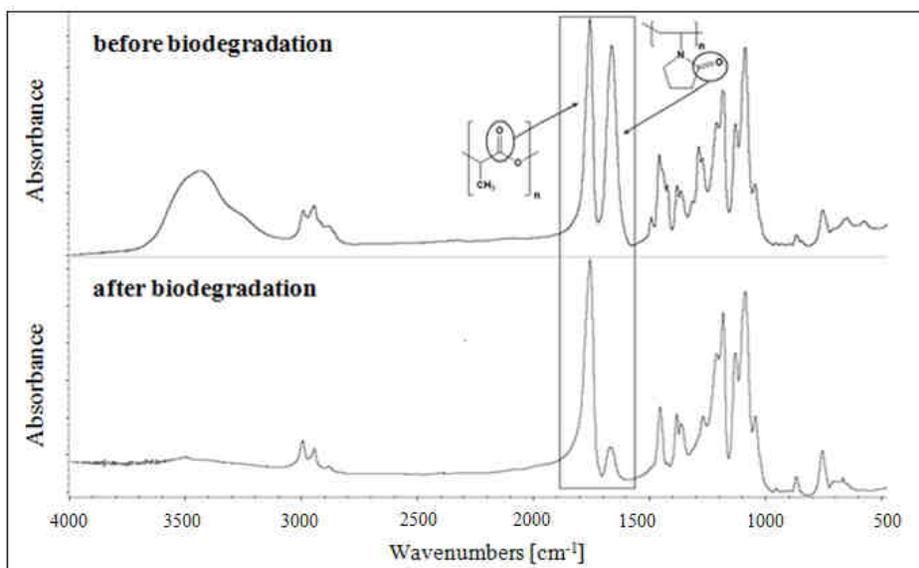


Fig. 4. IR-spectrum of film PVP/PLA ($500\text{-}4000\text{ cm}^{-1}$) before and after experiment in soil environment (70 days)

The Figure also gives the typical spectrum of PLA functional groups: a C=O group at 1760 cm^{-1} and the next characteristic peaks proving the presence of a -CH_3 group and a -C-O-C- bond are at 1454 cm^{-1} and at 1274 cm^{-1} , respectively. However, no significant changes are observed for PLA after the experiments.

These results support the theory that PVP was removed during the biodegradation experiments. Nevertheless, the dips do not stem from the process of biodegradation. It is almost certain that PVP fraction was eluted into the experimental environment, due to its easy solubility. This fact also suggests a low production of CO₂ in respirometry tests.

We also subjected the films to SEM in order to better understand the properties of the films. The images of surface of the films contained additives taken by SEM are given in Figure 5. The effect of additives on the structure is perspicuous, however, does not lead to improvement of biodegradation of this system.

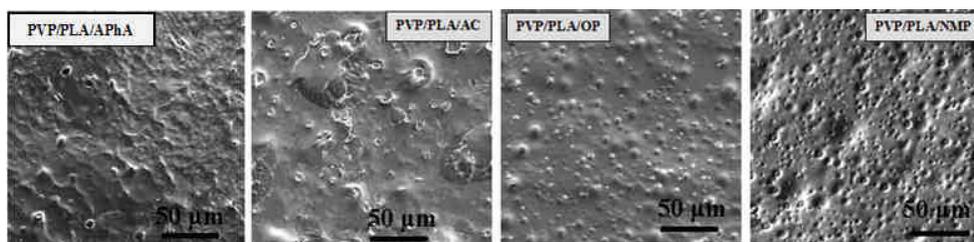


Fig. 5. SEM - image of surface of PVP/PLA/additives

The surface of the film without additives gives us Figure 6a. As can be seen, there is a visible heterogeneity of PVP/PLA system (a sharp interface between the particles of PVP and the PLA matrix), probably caused by the immiscibility of these substances [28]. This heterogeneity of the films explains some small discrepancies among the results of the particular tests.

Referring to the publication of de Paula et al., who state a higher rate of PVP/PLA system biodegradation [18], the biodegradation of only PLA is more probable. Apparently, pores remained (Figure 6b) in the PLA matrix, after the removing of PVP portion, whereby the surface of PLA could become more accessible to microbial cultures.

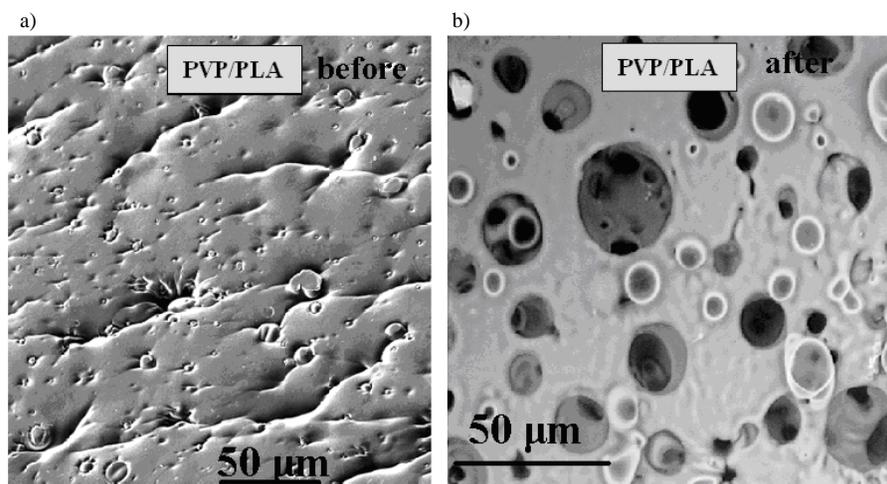


Fig. 6. SEM images of surface of PVP/PLA: a) before and b) after biodegradation test in water environment under aerobic conditions (45 days)

Removal of PVP was also visible with the naked eye, because the films lost transparency and their original yellow tinge, which is characteristic for materials prepared from PVP [29]. Figure 7 gives an optical view of films before and after biodegradation in soil environment.

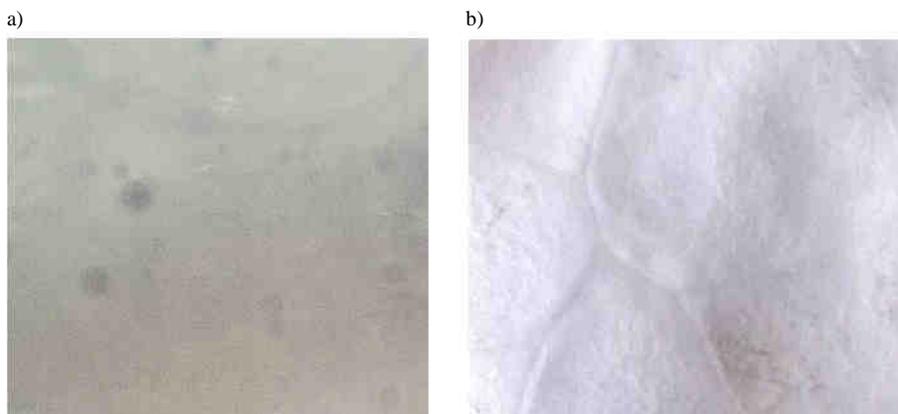


Fig. 7. An optical view of films PVP/PLA: a) before and b) after biodegradation test in soil environment (70 days)

Conclusions

Our research dealt with biodegradation of polymer films prepared from PVP/PLA (50/50) by the method of casting. We also tested how to support their biodegradation by addition of cosubstrates (NMP, OP, AC, APHA).

A microbial decomposition was simulated in soil and in water (aerobic and anaerobic) environment. Overall, the results are not directly comparable, because of the distinctive features of each environment. We noticed a slow rate of decay; nonetheless, it primarily corresponded to biodegradation of additives. We would like to point out that this research concentrated mainly on biodegradation, it has not studied physical and chemical properties of these materials in detail.

According to the de Paula's study et al. [18], where a higher rate of biodegradation of PVP/PLA system was noticed, the biodegradation of PLA would be more likely, because of more accessible surface after the removing of the PVP portion. Unfortunately, we are unable to determine from this data whether PVP or PLA was actually decomposed.

Further research in this area might show more complex results of biodegradation of other materials based on PVP.

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References

- [1] Robinson BV, Sullivan FM, Borzelleca JF, Schwartz SL. A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone. Chelsea: Lewis Publishers, INC; 1990.
- [2] Sedlarik V, Saha N, Kuritka I, Saha P. *Polym Compos.* 2006;27:147-152. DOI: 10.1002/pc.20197.
- [3] Julinova M, Slavik R, Kupec J, Vaskova M. *Ecol Chem Eng S.* 2013;20:199-208. DOI: 10.2478/eces-2013-0015.
- [4] Julinova M, Kupec J, Houser J, Slavik R, Marusincova H, Cervenakova L, et al. *Water Environ Res.* 2012;84(12):2123-2132. DOI: 10.2175/106143012 X13373575830999.
- [5] Julinova M, Kupec J, Alexy P, Hoffmann J, Sedlarik V, Vojtek T, et al. *Polym Degrad Stab.* 2010;95(2):225-233. DOI: 10.1016/j.polymdegradstab.2009.10.008.
- [6] Vaclavkova T, Ruzicka J, Julinova M, Vicha R, Koutny M. *Appl Microbiol Biotechnol.* 2007;76(4):911-917. DOI: 10.1007/s00253-007-1062-1.
- [7] Hirokishi S, Serpone N, Yoshizawa S, Hidaka H. *J Photochem Photobiol A.* 2001;138:69-77. DOI: 10.1016/S1010-6030(98)00408-0.
- [8] Loraine G. *Water Environ Res.* 2008;80:373-379. DOI: 10.2175/106143008X266779.
- [9] Giroto J, Costa A, Nascimento C, Guardani R. *Chem Eng Process.* 2008;47:2361-2369. DOI: 10.1016/j.ccep.2008.01.014.
- [10] Abd El-Mohdy HL, Ghanem S. *J Polym Res.* 2009;16.1:1. DOI: 10.1007/s10965-008-9196-0.
- [11] El-Houssiny A, Ward A, Mansour SH, Abd El Mesieh S. *J Appl Polym Sci.* 2011;124:3879-3891. DOI: 10.1002/app.35483.
- [12] Roy S, Saha N, Kitano T, Saha P. *Carbohydr Polym.* 2012;89:346-353. DOI: 10.1016/j.carbpol.2012.03.008.
- [13] Hu Y, Jiang Z, Chen R, Wu W, Jiang X. *Biomacromolecules.* 2010;11:481-488. DOI: 10.1021/bm901211r.
- [14] Kim GM, Le KH, Giannelli SM, Lee YJ, Rainer A, Trombetta M. *J Mater Sci Mater Med.* 2013;24:1425-1442. DOI: 10.1007/s10856-013-4893-6.
- [15] Hurst GA, Novakovic K. *J Materials Res.* 2013;28:2401-2408. DOI: 10.1557/jmr.2013.134.
- [16] Chao YC, Su SK, Lin YW, Huang KS. *J Environ Polym Degrad.* 2012;21:160-165. DOI: 10.1007/s10924-012-0450-5.
- [17] Soroory H, Mashak A, Rabimi A. *Iranian Polymer J.* 2013;22:791-797. DOI: 10.1007/s13726-013-0178-7.
- [18] De Paula E, Mano V. *Quim Nova.* 2012;35:1084-1089. DOI: S0100-40422012000600003.
- [19] Papong S, Malakul P, Trungkavashirakum R, Wenunon P, Chom-in T, Nithitanakul M. *J Clean Prod.* 2014;65:539-550. DOI: 10.1016/j.jclepro.2013.09.030.
- [20] Rudeekit Y, Numnoi J, Tajan M, Chaiwutthinan P, Leejarkpail T. Determining biodegradability of polylactic acid under different environments. *J Metals Mater.* 2008;18:83-87. <http://www.material.chula.ac.th/Journal/v18-2-2/83-87%20RUDEEKIT.pdf>.
- [21] Tokiwa Y, Calabia BP. *Appl Microbiol Biotechnol.* 2006;72:244-251. DOI: 10.1007/s00253-006-0488-1.
- [22] Cheng HN, Gross RA. *Green Polymer Chemistry: Biocatalysis and Biomaterials.* Washington DC: Amer Chem Soc. 2010;1043:405-414. DOI: 10.1021/bk-2010-1043.fw001.
- [23] Okhita T, Lee SH. *J Appl Polym Sci.* 2005;100:3009-3017. DOI: 10.1002/app.23425.
- [24] ISO 17556:2012. Plasty - Stanovení úplné aerobní biodegradability materiálů z plastů v půdě měřením spotřeby kyslíku v respirometru nebo měřením množství uvolněného oxidu uhličitého. (Plastics - Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved). 2012. <http://seznamcsn.unmz.cz/>.
- [25] ISO 15705:2002. Jakost vod - Stanovení chemické spotřeby kyslíku (CHSKcr) - Metoda ve zkumavkách (Water quality - Determination of the chemical oxygen demand index (ST-COD) - Small-scale sealed-tube method). 2002. <http://seznamcsn.unmz.cz/>.
- [26] ČSN EN ISO 9408. Jakost vod - Hodnocení úplné aerobní biologické rozložitelnosti organických látek ve vodním prostředí stanovením spotřeby kyslíku v uzavřeném respirometru (Water quality - Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer). 2000. <http://seznamcsn.unmz.cz/>.
- [27] ČSN EN ISO 11734. Jakost vod - Hodnocení úplné anaerobní biologické rozložitelnosti organických látek kalem z anaerobní stabilizace - Metoda stanovení produkce bioplynu. (Water quality - Evaluation of the ultimate anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of the biogas production). 1998. <http://seznamcsn.unmz.cz/>.
- [28] Zhang G, Zhang J, Zhou X, Shen D. *J Appl Polym Sci.* 2003;88:973-979. DOI:10.1002/app.11735.
- [29] Wypych G. *Handbook of Polymers.* Canada: ChemTech Publishing; 2011.