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The effect of temperature and water absorption on enzymatic degradation of starch / polyvinyl alcohol blend film by α-Amylase

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Abstract

Thermoplastic starch (TPS) materials present several advantages to the plastic industry and when blended with other materials they can exhibit improved mechanical and moisture sensitivity properties compared to pure TPS materials. Further investigations on TPS: PVA blends are of particular interest due to their excellent compatibility and improved properties such as tensile strength, elongation, toughness and processability, predominantly due to an improvement in melt strength compared to pure TPS material. The aims of the study were to investigate the effect of varying polyvinyl alcohol content within the TPS blends on the rate and extent of starch enzymatic hydrolysis using enzyme alpha-amylase. Analyses the enzymatic degradation behavior of poly(vinyl alcohol) with starch was based on the determinations of Weight loss and the reducing sugars. The degraded residues have been examined by FT-IR spectroscopy and scanning electronic microscopy (SEM).

Keywords: TPS; PVA; Blend; Enzymatic degradation; α-Amylase.

1. Introduction

Biodegradable polymers have been a subject of interest for many years because of their potential to protect the environment by reducing non-biodegradable synthetic plastic waste [1-3]. It is quite important to develop some materials that can biodegrade to minimize the pollution. These materials not only provide the convenience for daily life but also minimize the impact to the environment after being used. In the long run, these materials will decompose into small environmentally friendly molecules and be handled in properly controlled environment [4, 5].

Biodegradation involves enzymatic and chemical degradation by living microorganisms [7, 8]. The applications of TPS materials are limited by poor mechanical strength properties and high moisture sensitivity [6, 9] Starch has been considered as a suitable source material because of its inherent biodegradability, ready availability, and relatively low cost. However, as compared to most petroleum-based polymers, the poor mechanical properties and relatively high hydrophilic nature of starch prevent its use in widespread applications [10]. For this reason, blends of starch with other biodegradable synthetic polymeric materials have been investigated for numerous packaging applications. Blends of starch with synthetic polymers (e.g. poly(vinyl

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alcohol), aliphatic polyesters, etc.) are prepared to achieve the desired performance for different applications. In such blends, the starch particles act as a promoter for plastic matrix biodegradation in applications such as drug delivery systems, hydrogels, bone cements and bone replacement/ fixation devices [11-14].

PVA, a polymer containing many hydroxyl groups, is water-soluble with excellent properties, such as low permeability and high water absorption capability, and is found in wide industrial and agricultural applications [11, 14-16]. Starch is a semicrystalline polymer stored in granules as a reserve in most plants. It is composed of repeating α-1,4- D glucopyranosyl units: amylose and amylopectin. The amylose is almost linear, in which the repeating units are linked by α (1–4) linkages; the amylopectin has an α (1–4)-linked backbone and ca. 5% of α (1–6)-linked branches. The relative amounts of amylose and amylopectin depend upon the plant source. Corn starch granules typically contain approximately 70% amylopectin and 30% amylase [12, 13]. Both fractions are readily hydrolysed at the acetal link by enzymes. The α-1-4-link in both components of starch is attacked by amylase; the α-1-6-link in amylopectin is attacked by glucosidases [13].

α-Amylase are endoamylases catalysing the hydrolysis of internal α-1,4-glycosidic linkages in the starch in a random manner (Fig.1).

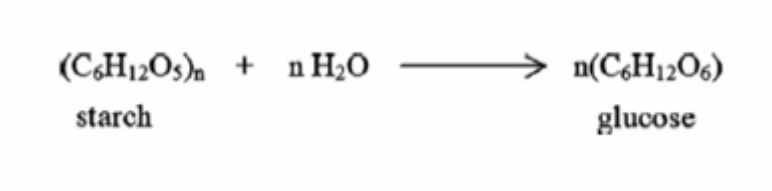


Fig. 1. Starch hydrolysis.

The microbial α-amylase for industrial purposes is derived mainly from Bacillus licheniformis, Bacillus amyloliquefaciens and Aspergillus oryzae. Polyvinyl alcohol was chosen because starch/ PVA blends have demonstrated excellent compatibility [17, 18]. Starch/ PVA blend plastics are one of the most popular biodegradable plastics, and are widely used in packaging and agricultural applications [19].

The biodegradable properties of these two polymers in common make them excellent pair for blending, and the water solubility of PVA makes it easy to mix evenly with the starch. All these lead to the extensive attention of the researches of starch/ PVA. In 1975, Griffin [20] proposed a method to increase the biodegradability by blending. Bastioli et al [21] reported that an amylose-PVA composite (PVA-starch blend) was very slowly biodegraded and that 75% weight loss required 300 days in a degradation test with activated sludge. The current paper studies the α-amylase action on starch/ poly(vinyl alcohol) blend film two temperature 25±1°C and 37±1°C for two concentration of enzyme. The modifications induced by the enzymatic treatment were evidenced by determination of mass loss, water absorption capacity, sugars released during biodegradation, as well as by UV spectroscopy and Total sugars were estimated by DNS method [22].

2. Experimental

2.1. Materials

Starch (ST) was provided by Merck company, and polyvinyl alcohol (PVA) with Mn=72000 and glycerol (Mn= 92/10, garde of pure 78%) purchased from Merck company. α-Amylase (source from Bacillus Subtilis) provided by sigma company.

2.2. Methods

The present work analyses the enzymatic degradation behavior blend of poly(vinyl alcohol) with starch was based on the determinations of mass loss and the reducing sugars. The blend have been prepared from 50 wt% PVA–50 wt% starch containing small amounts of plasticizers, stabilizers and destructuring agents. The biodegradation studies were carried out at 25±1°C and 37±1°C, pH= 7, using two concentration of α-amylase for 72h.

2.2.1. Enzymatic degradation

Each sample was placed in a vial filled with 20 mL of 0.1 mol L^{-1} phosphate buffer, pH 7.0, with 50 μL from 5×10^{-3} mol L^{-1} $CaCl_2$ at containing two concentration of α-amylase 1 mg and 5 mg, for several hour at two temperature 25±1°C and 37±1°C . After 1, 2, 3, 5, 7, 9, 12, 24, 36, 48, 60 and 72 h, the samples were removed the samples from buffer solution, dry and weighed, respectively. The ratio of weight loss was determined by the following equation:

$$\% \text{ Weight loss} = (W_0 - W_1) / W_0 \times 100$$

where W_0 represents the initial weight of a specimen and W_1 is the weight of a specimen after degradation. Results are given in Table 1 and can be seen in Fig. 2.

Table 1
Results Weight loss for TPS:PVA.

Time (hours)	Enz. 5 mg. Tem. 37 °C	Enz. 5 mg. Tem. 25 °C	Enz. 1 mg. Tem. 25°C	Enz. 1 mg. Tem. 37 °C
1	28.78	38.65	10.45	10.09
3	35.23	43.97	14.92	23.93
5	38.94	49.73	18.48	24.03
7	42.09	57.43	25.39	33.13
9	50.78	58.31	33.09	37.87
12	51.49	57.24	34.14	33.95
24	52.32	56.53	34.96	35.87
36	50.87	56.09	35.24	34.83
48	53.32	57.42	34.28	35.9
60	51.4	55.68	33.14	34.07
72	54.51	57.1	34.01	34.96

2.2.2. Water Uptake

The water absorption capability Pre-dried samples were weighed for the dry weight, and then placed in a bath in distilled water at room temperature. After 1, 5, 10, 15, 22, 27, 32, 37, 44 and 50 h, the samples were removed from distilled water and weighed. The water absorption capability was calculated with the equation below:

$$\% \text{ Water uptake} = (W_{wet} - W_{dry}) / W_{dry} \times 100$$

Where W_{wet} represents the weight of the wet specimen and W_{dry} represents the weight of the dry specimen. Results are given in Table 2 and can be seen in Fig. 3.

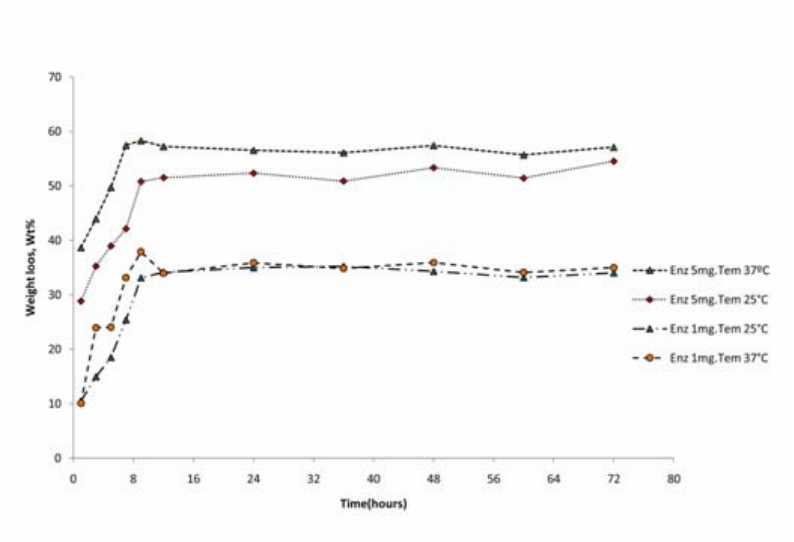


Fig. 2. Weight loss vs degradation time for TPS:PVA.

Table 2
Results Water adsorption for TPS:PVA.

Time (hours)	1	4	9	23	27	32	46	50
W_{wet}	0.507	0.514	0.551	0.606	0.603	0.611	0.623	0.675
W_{wet}-W_{dry}/W_{dry}×100	407	414	451	506	503	511	523	575

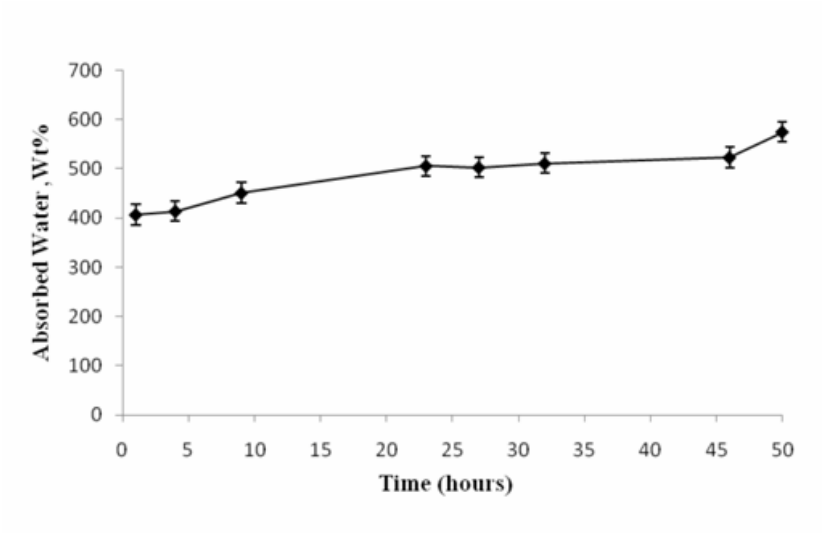


Fig. 3. Water adsorption vs time for TPS:PVA.

2.2.3. Detection of reducing sugars

The Nelson-Somogyi method is one of the classical and widely used methods for the quantitative determination of reducing sugars. For sugar estimation an alternative to Nelson-Somogyi method is the dinitrosalicylic acid method-simple, sensitive and adoptable during handling of a large number of samples at a time. The reducing sugars in the degradation solutions were quantified by the dinitrosalicylic acid method: 1 mL of reagent DNS was added to 1 mL of the sample to be analysed [22] using 1 mg mL^{-1} glucose stock solution as a standard. At

the same time, the blank was prepared using 1 mL of control sample. The mixture was heated at 90-100 °C for 10 min. After cooling to room temperature, 5 mL of distilled water was added, and the absorbance at 540 nm was measured in the. The respective carbohydrate concentration was obtained by comparison with a standard curve. Results are given in Table 3 and can be seen in Fig. 4.

Table 3
A summary of the rates of glucose production from substrate.

	Rate ($\mu g\ mL^{-1}h^{-1}$)	R^2
Enz. 5mg.Tem 25°C	31.46	0.992
Enz. 5mg.Tem 37°C	35.96	0.941
Enz. 1mg.Tem 25°C	29.01	0.975
Enz. 1mg.Tem 37°C	32.84	0.964

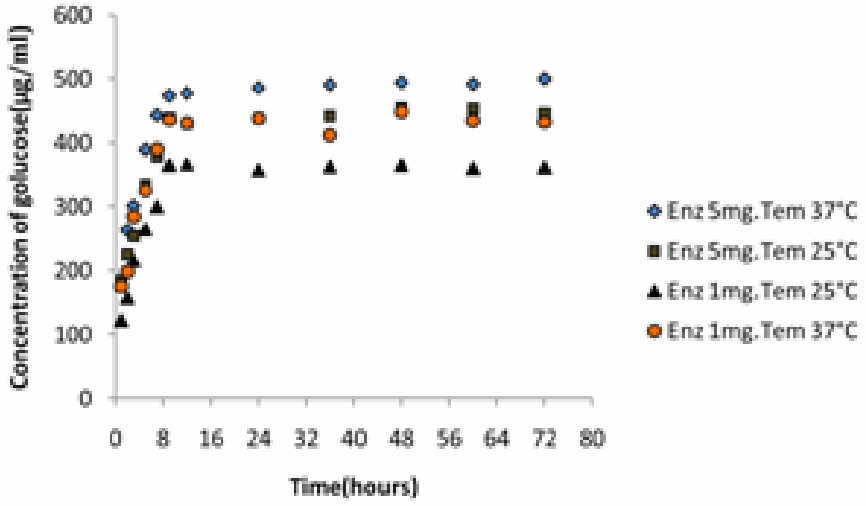


Fig. 4. Concentration of glucose produced for TPS:PVA blend film due to the enzymatic attack by α-amylase.

2.2.4. Scanning electronic microscopy (SEM)

The morphology of the surface of the films, before and after biodegradation, was investigated using a scanning electronic microscope of XL30 type (Netherland). The films were covered with pure metalic Ag. The laying down of Ag was carried out using evaporation of the metal under a high vacuum, to give a thickness of around 100 °A, Fig. 5.

2.2.5. FT-IR spectra

Infrared spectra with a resolution of 4 cm^{-1} of the samples as KBr pellets were recorded by Shimaszu FT-IR RF50 spectrometer.

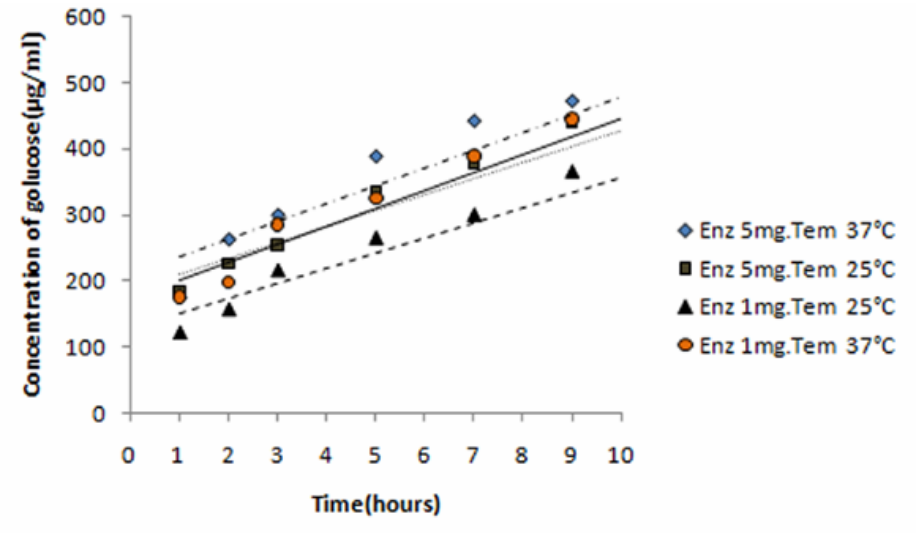


Fig. 5. Concentration of glucose produced for starch/PVA blend film in the first 9 h of enzymatic degradation due to the action of α-amylase**.**

3. Results and discussion

Degradability of polymers is a critical functionality for their application. Currently, no official standard method was established in determining biodegradability of polymers. The enzyme method [23] the microbiological method [24] and the soil burial method [25, 26] have been used by different researchers. Moreover, the biodegradability was also recorded by diverse indexes even in the same method [27] The present study shows the role of α-amylase in TPS:PVA degradation. Bajpai and Shrivastava [28], who studied the biodegradation of carboxymethyl-cellulose/ starch blends, found that, at small amounts of starch in the blend, a high percent of mass loss occurred while, at high starch contents, the mass loss was lower. This variation was explained, in the first case, by the increase of the number of starch molecules contacting the α-amylase, so that the amount of degraded starch was higher. At high starch contents, the material becomes much more compact, which hinders the α-amylase diffusion in the polymer film.

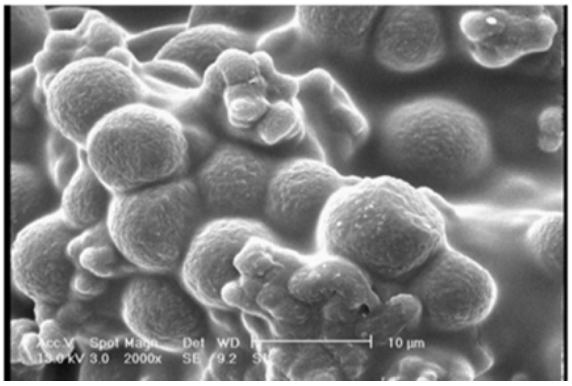
3.1. Weight loss and water uptake

The water absorption capacity and the degradability are the most important properties for biodegradable materials [29] The water absorption capacities of the TPS:PVA film at two temperature 25±1°C and 37±1°C were found to have significant difference. This was consistent with the results of Follain et al [30]. Increase of concentration of enzyme leads to the increase of both mass loss and reducing sugar Fig. 2 and Fig. 4, show clearly that degradation is much more pronounced when the water sorption is high. A comparison between the variation of the mass loss and water sorption capacity with respect to enzyme content show clearly that degradation is much more pronounced when the water sorption is high. Starch/ PVA exhibit both a high water sorption and the most significant mass loss.

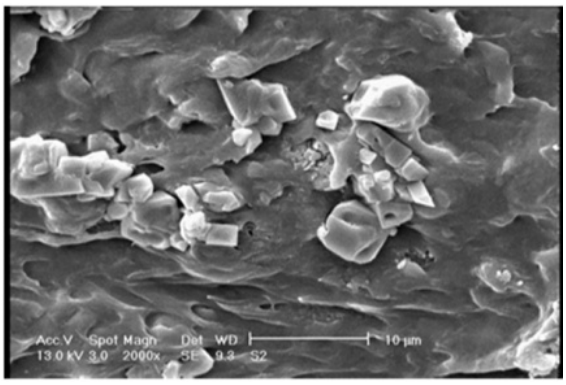
3.2. Rate and extent of glucose production

The rate and extent hydrolysis by the action of α-amylase was measured using the DNS method glucose assay of films of varying starch concentration. The production of glucose was used as a measure of starch hydrolysis. Fig. 4. shows the extent of glucose over a 72h hydrolysis time for substrate. Reducing sugars in the degradation solution the amount of reducing sugars in the degradation solutions, reduced by dinitrosalicylic acid, increased since the beginning until

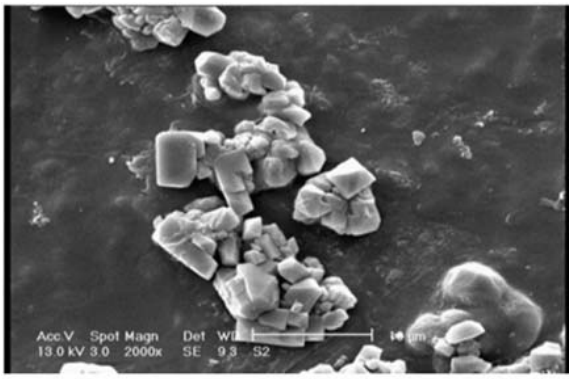
the end of the assay the relative amount of reducing sugars in the degradation solutions in similar assays without enzymes was about 100 times lower.



(a)



(b)



(c)

Fig. 6. Microscopic views of TPS:PVA degradable films: (a) undegraded; (b) starch/ PVA degraded due to the action of α- 1mg amylase for temperature 37 °C; (c) starch/ PVA with due to the action of 5mg α-amylase for temperature 37 °C.

One of the routes of biodegradation is by hydrolysis, and the enzymatic hydrolysis of starch is accompanied by the release of glucose. Fig. 5. shows the release of glucose (μg mL^{-1}) during exposure to α-amylase, refer to Table 3 by assuming a linear relationship between the concentration of glucose and time for the first 9 h of hydrolysis. The rate of starch hydrolysis was most rapid for the substrate at high temperature and concentration of enzyme. The amount of free glucose increased with time for the blend TPS:PVA blend showed a peak release of

glucose at 9 h, followed by a decline. Apparently, the PVA have a stabilizing effect against the enzymatic attack, even after increasing the content of insoluble fraction.

3.3. Scanning Electronic Microscopy (SEM)

Several optical microscopy images of TPS:PVA blend are given in Fig. 6. One may observe that the films are considerably destroyed, although during degradation, a much more stable fibrillar fraction is revealed.

3.4. FT-IR spectra

The FT-IR spectrum of undegraded and degraded blend in the presence of α-amylase is shown in Fig. 7. In this study attention was focused on the range in the absorption pattern in six main regions: (a) stretching vibration of the O-H group between 3436 and 3455 cm^{-1}; (b) Stretching vibration of the C=O at about 1740 cm^{-1} ; (c) Stretching vibration of the C=C at about 1638 cm^{-1}; (d) Stretching vibration of the C-O at about 1237 cm^{-1}; (e) Stretching vibration of the C-H at about 1454 cm^{-1}; (f) Stretching vibration of the COO^- group between 1500- 1600 cm^{-1}.

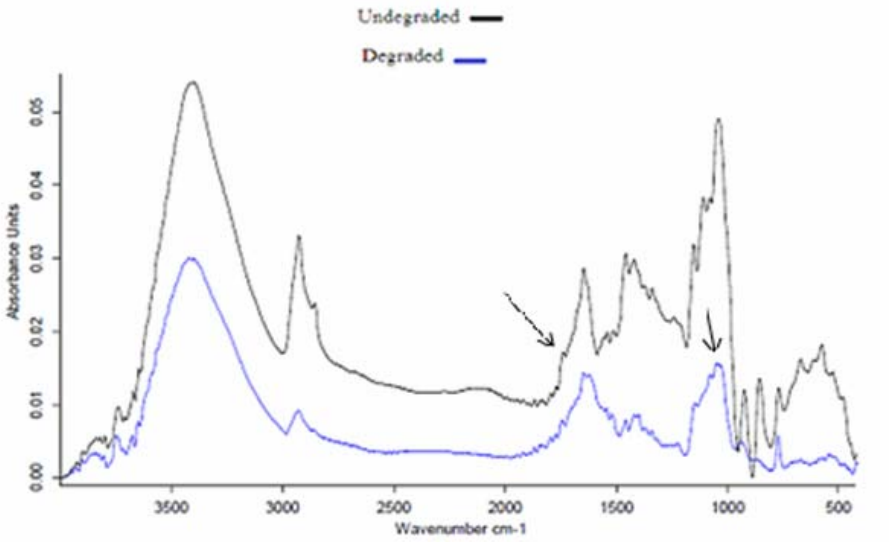


Fig. 7. FT-IR spectra of the TPS:PVA (undegraded and degraded).

The broad band in the region of 3430 cm^{-1} is due to the hydroxyl stretching vibration and the band in the region of 2929 cm^{-1} is due to CH_2 asymmetric and symmetric stretching vibration [25, 31] The peak at 1454 cm^{-1} was assigned to CH_2 bending vibration [27]. The absorbances at 1115, 1163 and 1026 cm^{-1} are more sensitive to the conformational changes produced during degradation processes, indicating a short range order and helicity changes when crystallinity and molecular orientation are lost [25] After degradation with α-amylase, the intensity of the peak at 1115 and 1040 cm^{-1} decreased, indicating the action of α-amylase in cleaving the glycosidic linkages of starch [32].

4. Conclusions

The present study shows the role of α-amylase and temperature in TPS:PVA blend degradation. The PVA content significantly impacted on the rate of starch solubilistion. The decrease of the degradation rate observed in the final stage can be explained to the lower degradability of the PVA domains that remain in the material. After 10-72 hour, the variation is almost negligible, nearly zero, as no saccharides and other compounds leached to the solution, as

demonstrated before. The reduction of the degradation rate is also influenced by the water uptake ability of these polymers.

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