Polymer Degradation and Stability
July 2014, Volume 105, Pages 237-247
http://dx.doi.org/10.1016/j.polymdegradstab.2014.04.026

Seawater accelerated ageing of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

Morgan Deroiné[a], Antoine Le Duigou[a], Yves-Marie Corre[a], Pierre-Yves Le Gac[b], Peter Davies[b], Guy César[c], Stéphane Bruzaud[a, *]

[a] Université de Bretagne-Sud, Laboratoire d'Ingénierie des Matériaux de Bretagne (LIMATB), Rue de Saint Maudé, 56321 Lorient Cedex, France
[b] IFREMER, Marine Structures Group, Centre de Bretagne, BP 70, 29280 Plouzané, France
[c] Université de Bretagne-Sud, SERPBIO, Rue de Saint Maudé, 56321 Lorient Cedex, France

*: Corresponding author : Stéphane Bruzaud, tel.: +33 2 97 87 45 84 ; fax: +33 2 97 87 45 88 ;
email address : stephane.bruzaud@univ-ubs.fr

Abstract:

The aim of this study is to establish a baseline for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) lifetime prediction in a marine environment, by means of mechanical and physico-chemical characterization of specimens immersed in continuously renewed and filtered natural seawater in the laboratory at different temperatures (4, 25 and 40°C). Samples were also aged at sea in Lorient harbour in order to compare laboratory and natural degradation mechanisms and kinetics. Due to its morphology, hydrolysis of PHBV in natural seawater is quite slow, and samples were observed to undergo preferentially an enzymatic surface degradation. Increasing the aging temperature in the laboratory promotes the water uptake and causes hydrolysis. As two degradation mechanisms occur in parallel, the choice of test conditions is critical, and the lifetime of PHBV in a marine environment is difficult to predict accurately.

Keywords : Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) ; accelerated ageing ; hydrothermal degradation ; seawater

1. Introduction

Over the last decade, development of biodegradable polymers has been evolving continuously [1,2]. Recently, pollution of nature by plastics has resulted in a growing awareness of this environmental concern, and enhanced the interest for developing biodegradable polymers or polymers for which the lifetime is controlled. Large quantities of plastic waste are discarded into the oceans by winds, rivers or maritime activity [3,4]. Besides their petrochemical origin, a major drawback of these conventional plastics is their end of life management. Indeed, the high ratio between their lifetime and their period of use generates an accumulation of waste which progressively fragments. Harmful consequences for marine wildlife, and increasing dangers for humans are now well established [5,6].

Polyhydroxyalkanoates (PHA) are microbial synthetized polyesters [7] which have received considerable attention as biodegradable alternatives to conventional plastic. From a life cycle point of view, they are interesting because they are produced from renewable resources and they are also biodegradable [8]. Overall environmental impacts are lower than those of traditional polyolefins but some aspects such as the use of chemical products for the cellular extraction or for the purification still require improvement [9,10].

PHA has many other advantages like biocompatibility, thermoplastic processing capacity and properties similar to various synthetic thermoplastics. PHA can be produced using organic waste and is not in competition with food production, unlike PLA. Moreover, the thermal and mechanical properties of PHA can be controlled by varying the amount of valerate in copolymers [11,12]. In this study, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), a particularly promising PHA copolymer [13], is studied.

A remarkable advantage of these polymers is their biodegradability in various environments: in soil, in compost, in fresh water and more especially in various marine environments like mangrove in tropical coastal waters in the Caribbean sea [14,15], South China sea [16,17], the

Pacific ocean off the Japanese coast [12,18–20] or in Baltic sea water [21]. The PHA degradation rate is known to be influenced by different factors especially those related to the environment such as weather conditions, microbial population [18], pH [22], temperature, chemical composition of the PHA, the polymer geometry (surface area) and its processing induced morphology [17].

A large number of studies have examined the identification of bacteria capable of degrading or ingesting PHBV but mainly focusing on biodegradation mechanisms [12,17,18,23,24]. Few studies have compared the influence of different kinds of seawater on the degradation, and very few used temperature to accelerate ageing of PHBV. Also the majority of studies have focused on very thin film specimens (a few µm thick) and characterizations are limited. Moreover, PHBV lifetime in marine environment is not clearly established.

In the present study, ageing of PHBV specimens in seawater, natural or filtered and renewed, was investigated at different temperatures over a period of 360 days. In order to establish a baseline for degradation mechanisms, several mechanical and physico-chemical characterizations were carried out using gravimetry, surface roughness measurement, scanning electron microscopy (SEM), tensile tests, steric exclusion chromatography (SEC) and differential scanning calorimetry (DSC). PHBV ageing was followed at different temperatures in order to evaluate the validity of a lifetime estimation based on the Arrhenius relationship.

2. Experimental

2.1. Materials

PHBV with a molar ratio of HB to HV of 92:8 and commercialized in pellet form under the name ENMAT Y1000P was supplied by Tianan Biological Materials Co. Ltd. (China). According to the manufacturer, PHBV has the following properties: density = 1.25 $g.cm^{-1}$, T_g

≈ 4°C, T_m = 175°C and an average molecular weight $\overline{M}_w$ ≈ 400 kg.mol^{-1}. This grade has been comprehensively characterized in a recent paper [25].

2.2. Preparation of tensile specimens

PHBV pellets were dried at 50°C under vacuum for 24h before processing. For the preparation of dog-bone specimen, pellets were injected using a Battenfeld HM 80/120 press. The hopper temperature was 160°C and the profile was the following: 160-165-170-170°C. Injection pressure was 1200 bars and the mould temperature was kept at 35°C. All parameters were kept constant throughout the injection moulding process. PHBV was injected in a mold designed to produce standard specimens, 180 x 10 x 4 mm geometry (specimen 1A according to NF EN ISO 527).

2.3. Ageing conditions

Ageing tests were performed under two conditions: immersion in natural seawater and in filtered and renewed seawater for one year. Accelerated ageing tests were carried out in seawater baths (60L) at 4, 25 and 40°C, with filtered and continuously renewed seawater pumped from the Brest Estuary (France). Ageing experiments must be performed at temperatures superior to the PHBV glass transition temperature (T_g) to be sure that the polymer is in the same morphological state. Natural ageing tests were also performed in the Lorient harbour (France). Characteristic parameters of seawater between Brest and Lorient were substantially the same during the study, from March 2012 to March 2013, and are summarized in Table 1. Water temperature varied from 8.6 to 19.8°C. Lower temperatures are well-known to slow down the bacteria activity. Water pH was constant around 8 during the year except in October with a peak at 8.5. Salinity and oxygen contained in the seawater were in the standard average range.

Table 2 shows the mean number of colony-forming unit (CFU) determined for seawater samples taken in the four different areas. The bacterial contribution is an essential parameter for PHA studies. A series of seawater dilutions was mixed with marine agar and incubated at 25°C for 24h. After the necessary incubation period, the colony counting was performed for each petri dish containing less than 300 colonies. The value in the initial seawater is obtained by taking into account the dilution ratio. The CFU number is most important in natural seawater. For the baths at different temperatures, seawater was filtered and continuously renewed. The CFU number is lower at lower temperature, and difference between 4 and 25°C is not significant in microbiology. Filtration can prevent specific types of microorganisms from entering the baths but the temperature increases the development of thermophilic bacteria in the bath, with a high value of CFU number at 40°C. Moreover, the renewal of seawater increases the time for microorganism to colonize surfaces.

Table 1 - Table 2

2.4. Characterization techniques

2.4.1. Water uptake

A set of dog-bone specimens immersed in water were removed, washed several times with distilled water, wiped and weighed at room temperature (23°C and RH = 50%) on a weighing machine with a precision of 0.1 mg. The percentage gain at any time *t*, M_t, was determined by Eq. (1):

$$M_t(\%) = \frac{W_t - W_0}{W_0} \times 100 \quad (1)$$

where W_t and W_0 denote, respectively, weight of sample after exposure to water and weight of dry material before immersion.

A Dynamic Vapour Sorption (DVS 5000, TA Instruments) device was used to evaluate water diffusion coefficient, in a 160 µm thick film cut directly in the dog-bone specimen, at different Relative Humidity (RH) for temperatures ranging from 20 to 50°C. Although water diffusion coefficients have been measured under conditions different from those of ageing, these values are still interesting because the water diffusion kinetics in a polymer are not greatly affected by the value of partial pressure of the water in the external environment.

The weight gain resulting from moisture absorption can be modelled in terms of two parameters, the water diffusion coefficient D and the maximum moisture content M∞, are given by Fick's first law, Eq. (2):

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum \frac{1}{(2i+1)^2} \exp\left(-\frac{D(2i+1)^2 \pi^2 t}{e^2} \right) \quad (2)$$

where e is the thickness of the sample. D can be calculated from the initial linear portion of the absorption curve. For $M_t/M_\infty \leq 0.5$ the function $M_t = f(\sqrt{t})$ is linear and the Fickian diffusion coefficient D is determined by Eq. (3):

$$D = \frac{\pi}{16} \frac{e^2}{t} \left(\frac{M_t}{M_\infty} \right)^2 \quad (3)$$

Commonly, D value is constant whatever the relative humidity and extrapolation for immersion in water is then possible.

2.4.2. Weight Change

Following weight is a useful method to characterize irreversible degradation. At selected immersion times, the percentage loss, at any time t, WL_t, was determined on drying dog-bone specimens by Eq. (4):

$$WL_t(\%) = \frac{W_o - W_d}{W_o} \times 100 \quad (4)$$

where W_0 and W_d, indicate, respectively, initial weight of materials prior to water exposure and weight of dried material (after immersion).

2.4.3. Surface roughness

Surface roughness was measured using a surface roughness tester (TR100, TIME group Inc, China). This device sweeps a surface and gives an average roughness (Ra) which corresponds to an average of surface irregularity (ridges and troughs) with a sensibility of 0.5 µm. After each weight measurement, tests were carried out on the dried sample and each specimen was measured in fifteen non-overlapping regions on the entire surface. The mean roughness was then determined.

2.4.4. Scanning electron microscopy (SEM)

Microscopy analyses were performed with a Jeol JSM 6460LV scanning electron microscope (SEM) to examine the surface and the fracture zone of the dry PHBV specimens. Prior to observation, the fracture surfaces of unaged and aged PHBV were prepared by fracture in liquid nitrogen. Then, specimens were sputter-coated with a thin gold layer by means of a sputtering apparatus (Edwards Sputter Coater) before examination.

2.4.5. Mechanical tests

Static tensile tests were carried out in an environmentally controlled laboratory, according to ISO 527 (23°C and 50% relative humidity) on an MTS Synergie RT1000 testing machine. The loading speed was 1 $mm.min^{-1}$ and an MTS extensometer was used to measure strain, over a 25 mm gauge length. Two types of tests were performed, one on wet specimens, one on specimens which had been dried. Drying conditions have been previously described (2.4.1 part). At least five specimens were tested for each condition, and the results were averaged arithmetically. The sections of aged samples were measured just before the test as they can evolve after removal from water.

2.4.6. Molecular weight measurement

Steric exclusion chromatography (SEC) was used to determine the evolution of molecular weight. The apparatus is equipped with a set of three columns: two ResiPore and one PL gel Mixed C (Polymer Labs.). The detection system is composed of a refractometer and a UV detector. Chloroform was used as eluent with a flow rate of 0.8 mL.min^{-1}. The elution profiles were analysed by the Empower GPC module software (Waters). Calculations are based on calibration curves obtained from polystyrene standards ranging from 200 g.mol^{-1} up to 6 × 106 g.mol^{-1}. The weight-average molecular weight ($\overline{M}_w$) and number-average molecular weight ($\overline{M}_n$) are obtained from the SEC analysis. The polydispersity index (PI) is calculated as $\overline{M}_w/\overline{M}_n$.

2.4.7. Thermal properties

Differential scanning calorimetry (DSC) analysis was performed on samples of about 10 mg, in standard aluminium pans, using Mettler-Toledo DSC882 equipment under a nitrogen atmosphere. Samples were taken in the middle of the dog-bone on a part of the section. Data were recorded at a heating rate of 20°C.min^{-1}. The samples were heated from 20 to 200°C and kept at 200°C for 3 minutes. The samples were then cooled to -20°C and finally a second heating scan was performed from -20 to 200°C to distinguish the reversible effects from permanent changes. The degree of crystallinity was determined by Eq. (5):

$$\chi = \frac{\Delta H_m}{\Delta H_{100\%}} \tag{5}$$

where ΔH_m (J.g^{-1} of polymer) are the melting enthalpy. $\Delta H_{100\%}$ is the melting enthalpy for PHBV of 100% crystallinity, taken to be 146 J.g^{-1} [27]. This value, found for PHB homopolymer by Barham *et al.*, is considered as a good approximation of the $\Delta H_{100\%}$ for PHBV sample, provided that the copolymers have a low percentage of hydroxyvalerate units [28].

3. Results and discussion

3.1. Water uptake

Fig. 1 shows the weight gain of PHBV as a function of $t^{1/2}$ (expressed in $H^{1/2}$) at different immersion temperatures in seawater. All the sorption curves follow the same trend with similar behaviour. An initial linear relationship between water uptake and square root of time is observed, followed by a saturation plateau. These observations indicate a typical Fickian behaviour. Value of M_∞ obtained for the equilibrium plateau region are summarized in Table 3. Increasing the temperature has an influence on values of the saturation plateau. This trend has already been observed for PHBV [29] and also in the case of PLA [30]. The water diffusion is influenced by the temperature and water absorption can induce minor chain cleavage which is sufficient to increase the number of hydrophilic sites in the polymer [31]. Moreover, water uptake can also cause swelling, thus facilitating the transport of water within the polymer.

Figure 1 - Figure 2 – Table 3

Commonly, water diffusion characteristics in polymers are usually characterized by weight gain measurements. In this study, PHBV samples have been immersed in seawater, natural or filtered and renewed, with the presence of marine microorganism as shown in Table 2, and weight loss can occur simultaneously with water uptake. To avoid this weight loss problem, DVS was employed and experiments were done at different temperatures in order to obtain an accurate diffusion coefficient. The M_∞ in immersion (RH = 100%) is not determined but one of the advantages of this technique is that diffusion coefficient must be constant whatever the relative humidity. An extrapolation was then possible to get diffusion coefficient value in immersion.

Table 4

At each RH increasing, the initial curves when $M_t/M_\infty \leq 0.5$ were used to determine the diffusion coefficients. Values of D for the different temperatures, according to Eq. (3), are given in Table 4 and the increasing of temperature has an effect on the diffusion coefficients. These values are similar to those found in the literature, especially at low temperature [29]. Moreover, a linear relation ($R^2 = 0.97$) is noted between lnD and the inverse of temperature (Fig. 2). Results show that diffusion is a thermally activated process, suggesting that these coefficients are governed by the Arrhenius law (Eq. 6):

$$D = D_0 \exp\left(-\frac{E_a}{RT}\right) \quad (6)$$

The activation energy E_a was obtained from the slope of linear fitting of lnD versus 1/T and was estimated at 71 kJ.mol^{-1} using DVS device. This value is higher than the few published values available, which are around 44 kJ.mol^{-1} [29]. In this study conditions and using the experimental data, the activation energy in seawater was estimated at 50 kJ.mol^{-1} (not shown here). This difference between E_a values could be explained by several reasons: the different kinds of aqueous environments, the grade of PHBV used or the processing conditions which modify the polymer morphology.

In summary, these results indicated an equilibrium water uptake in PHBV which is temperature dependent and diffusion coefficients also increase with temperature, following an Arrhenius relationship. The activation energy is constant over the temperature range studied and justifies one of the assumptions for using this approach for lifetime prediction.

3.2. Surface roughness

Modification of surface roughness could be the consequence of leached products produced by irreversible surface degradation due to polymer hydrolysis or surface erosion due to microbial attack. The influence of seawater immersion at different temperatures on surface roughness of PHBV specimens is presented on Fig. 3. Roughness change was higher for specimens aged in

natural seawater, with a maximum roughness of 8.3 µm observed after 270 days of immersion. In filtered seawater, at 4, 25°C and 40°C, the roughness increase was slower and corresponding values are respectively 2, 2.5 and 4.5 µm after 360 days of immersion.

Figure 3

SEM observations on the PHBV surface confirmed roughness analysis. Unaged PHBV shows a homogeneous and uniform surface (Fig. 4-1a). After one year in immersion in natural seawater, considerable changes occurred as shown on Figure 4-2a. The surface became totally heterogeneous and rougher with the apparition of holes and pores.

The surfaces of PHBV specimen aged in filtered seawater revealed different aspects according to ageing temperature. We noted the appearance of tiny holes at 4°C suggesting a relatively low roughness for this sample. The surface roughness was drastically modified with increasing ageing temperature since these holes became larger and deeper for the samples aged at 25 and 40°C (Fig 4-4a and Fig 4-5a). Another significant point was that the bulk of the PHBV retained the same appearance whatever the ageing temperatures or the kind of seawater, contrary to PLA which undergoes a core degradation [32,33].

Figure 4

Temperature variations influence the kinetics of erosion degradation but it is not the only influent parameter. Microbial population also appears to be an important parameter [34]. The degradation mechanism is enzymatic rather than hydrolytic and enzymatic degradation occurs from the surface with an erosion mechanism [7,24]. The enzymatic degradation of PHA is a heterogeneous reaction, which takes place in the presence of PHA depolymerase, involving two steps: the first step involves the adsorption of the enzymes on the surface by the binding domain of enzyme and the second step concerns the enzymatic cleavage of polymer chains by the active site of the enzyme [35]. Currently, only one degradation mechanism is highlighted and the traditional methodology for predicting lifetime can be used. However, micro-

organism species or concentrations are very water dependent. An accelerated factor will probably need to be taken into consideration otherwise microbial attack will be difficult to model.

3.3 Weight loss

Fig. 5 shows the evolution of weight loss as a function of time at different immersion temperatures. Weight loss of PHBV specimens was higher in natural conditions with a loss percentage of 8 wt.% after one year of immersion compared to 2 wt.% at 40°C in filtered seawater, 1 wt.% at 25°C and quasi negligible at 4°C. In the literature, on a sample aged in natural seawater with a similar thickness (2.5 mm), the percentage of loss of weight is quite similar: 12 wt.% [14].

Figure 5

Little effect of temperature was observed on the loss of weight or the bacterial number (Table 2), but the degradation seems to be rather dependent on the resident microbial populations [14]. For the baths at different temperatures, seawater was filtered and renewed and this could increase the time for microorganism to colonize surfaces. Nevertheless, if the number of bacteria is more important in the medium, statistically it is more likely that those specifically able to degrade PHBV will be present. In the literature, extracellular PHA depolymerases secreted from microorganisms have been already identified [15,36,37] and utilize the decomposed compounds as nutrients. A further analysis of bacteria must be considered in this case and biodegradation tests should be performed in order to confirm a microbial attack and a metabolized step.

The evolution of the roughness as a function of the weight loss is plotted on Fig. 6 and reveals a proportional relationship between these two parameters whatever the medium. Therefore, the weight loss mechanism involves uppermost layer degradation and “layer by layer” degradation (illustrated on Fig. 7) for the natural ageing, as previously observed [35,38].

Figure 6 – Figure 7

These results indicate degradation initially occurring on the surface of samples and that the measurement of roughness coupled with weight loss may provide a useful technique to follow degradation of PHBV in seawater immersion. Moreover, this degradation mechanism should be taken into account in any methodology to predict lifetime. Usually, the approach, based on an Arrhenius law, requires macroscopic indicators such as strain at break or stress at break. Weight loss following is probably not a judicious indicator for this approach, but it can provide an estimation.

3.3. Mechanical properties

Tensile tests were carried out on the aged PHBV samples in the wet state and after drying providing information on reversible and irreversible degradation mechanisms. Fig. 8 shows the tensile behaviour of PHBV samples after 12 months of immersion in seawater, natural or renewed and filtered. After one year of immersion at 4°C, ductility increased slightly for wet PHBV. An increase was also noted even after drying. At 25°C and for the natural ageing, mechanical behaviour was more brittle than that of the unaged PHBV after 12 months in immersion. In the dry state, PHBV partially recovered its initial properties. At 40°C, samples became completely brittle with a large decrease of strain and stress at break. In this case, even after drying, there was no return to the initial state which showed a typical irreversible degradation.

In this case, significant behaviour changes appeared only at 40°C and this was independent of the medium and the number/type of bacteria.

Figure 8

The figures 9, 10 and 11 represent the evolution of global mechanical properties after 12 months of immersion in seawater in different conditions, numerical values are presented in Table 5.

Figure 9 - Figure 10 - Table 5

Fig. 9 shows the evolution of the strain at break over the time and Fig. 10 exhibits the evolution of stress at break during all the ageing. Weight loss of PHBV specimens, previously discussed, led to a decreasing section over time. The global cross section evolution was taken into account for the calculation of stress at break, a comparison between the initial section and section after immersion, is presented in Table 5. Strain at break may be more reliable as it does not depend on PHBV cross section.

After immersion at 4°C, strain and stress at break remained constant and showed a slight increase after drying. For samples aged in natural seawater or at 25°C, the changes observed were similar. After a slight increase, failure strain decreased very slowly in the wet state but remained constant after drying. Stress at break remained constant for these two ageing conditions. For wet tests after immersion at 40°C, an initial increase in the failure strain was observed after one month, presumably due to plasticization, followed by a constant decrease until the end of the study as hydrolysis occurred. On dried specimens, failure strain decreased continuously with immersion time. The percentage loss in properties was more than 50% after one year in immersion at 40°C. The decrease in stress at break, on the wet state, was more pronounced after 6 months of immersion, reaching 35% after one year. However, the reduction was less clear after one year in the dry state test with only 15% loss (considering the initial section, the loss is around 20%).

We can conclude that the differences in the mechanical properties obtained from calculations using the either the initial cross section or the section at a given moment are not significant even though weight loss is important.

Figure 11

Young's modulus evolutions are shown on Fig. 11. At low temperature, stiffness was almost constant with ageing time whereas after immersion at 40°C, it increased during the ageing. In the dry state, Young's modulus tended generally to increase. On samples aged in air, the same trend was observed: mechanical properties were not stable with time and tended to increase [25].

Despite the loss of weight previously observed for the samples, global mechanical properties remained constant until 25°C, as already found in previous studies [19]. Moreover, after one year at 4°C, mechanical properties of PHBV tended to evolve. The increase in temperature promoted a premature loss of properties, especially at 40°C with an irreversible degradation of PHBV specimens.

3.4. Molecular weight analysis

Changes in molecular mass of PHBV specimens after marine exposure are shown in Fig. 12, and values after one year of immersion are presented in Table 6.

Figure 12 – Table 6

For the lower temperature, there was no significant difference between the natural ageing and the ageing at 4 and 25°C in renewed and filtered seawater. $\bar{M}_w$ decreases over time to a final reduction of 30%. At 40°C, the weight average molecular weight decreased quite drastically through the first 6 months corresponding to a reduction of about 60%, and remained relatively constant during the last 6 months of immersion. At high temperature, chain breakage was

more extensive and ester bond hydrolysis was the main degradation mechanism. A similar trend has been already observed for polyhydroxyalkanoates in a previous study of natural ageing [16], for which the number average molecular weight, $\overline{M}_n$, decreased quite drastically through the first week of the study but remained almost constant for the following weeks.

Despite the 30% molecular weight reduction, mechanical properties were relatively constant for the ageing at lower temperatures. These results showed that chain breakage appeared but that there was only a minor influence on mechanical properties, with a slightly decrease of strain at break. This could be attributed to the high molecular weight for residual PHBV samples even after one year. We assume that a molar mass threshold exists, above which mechanical properties were not altered. Indeed, Fig. 13 shows the relation between mechanical properties (σ_b and ε_b) and molecular weight ($\overline{M}_w$). Two trends were highlighted: the stress at break was relatively constant over time while the strain at break seemed to be more dependent on molecular weight, especially at 40°C. For the points corresponding to the ageing at this temperature, the molar mass threshold may be reached with a critical molecular weight, corresponding to an "end of use" criterion, close to $\overline{M}_w$ = 120 000 g.mol^{-1}.

Figure 13

The relation between water uptake and molecular weight is presented on Fig 14 and there is a clear link between these parameters. More extensive chain breakage occurred when PHBV samples absorbed more water. In contrast, no relationship was established between loss of weight and molecular weight, as shown in Fig 15, similar findings were reported previously [16,39].

Figure 14 – Figure 15

Generally, the PHBV hydrolysis is slow because PHBV is highly crystalline and hydrolysis occurs preferentially in amorphous regions [40]. Molecular weight measurements highlighted a second degradation mechanism, the slow hydrolysis of esters bonding. This mechanism must be taken into account in lifetime prediction as it will add to the first mechanism identified previously, enzymatic degradation on the surface. The co-existence of two mechanisms makes the assumptions of an Arrhenius prediction methodology invalid.

3.5. Thermal properties

Modification to polymer morphology due to the degradation can be revealed by DSC analysis. Table 7 summarizes the thermal properties of PHBV after 12 months of immersion in natural seawater and at 4, 25, and 40°C in renewed and filtered seawater.

Table 7

During the first heating, no change was observed for the melting temperature for natural ageing and for lower temperatures, i.e. 4 and 25°C. A small decrease in melting temperature was observed at 40°C for both first and second scans which emphasized a higher degradation rate compared to other temperatures and medium, according to results obtained using SEC. The decrease in melting temperatures with increasing ageing temperature was attributed to the formation of PHBV chains with lower molecular weight.

A slight increase of the crystalline index χ_c was noticed especially for samples aged naturally and in filtered seawater bath at 40°C. This recrystallization phenomenon may explain the evolution of Young's modulus. A slight evolution of mechanical properties at lower temperature can be explained by a secondary crystallization through the amorphous spherulitic lamellae [30]. Another possible explanation is that microstructural changes occured when the immersion temperature in the bath was 4°C, which is close to the T_g of

PHBV. Lifetime prediction could be further complicated if PHBV morphology evolves over immersion time.

4. Conclusions

In this work, natural ageing at sea and accelerated laboratory ageing of PHBV have been performed. Ageing was accelerated by the temperature and the limits to lifetime prediction were discussed. Different characterization techniques have been used in order to follow the degradation and the different mechanisms have been identified.

One of the first results obtained in this study is that PHBV specimens degraded more rapidly under natural conditions at sea with a higher loss of weight, without significantly losing their mechanical properties. The development of polymer materials which undergo degradation under natural seawater conditions could contribute to reducing pollution problems caused by marine plastic waste.

The traditional laboratory method to accelerate polymer ageing, by increasing the temperature, in this case while increasing diffusion rate resulted in a much lower loss of weight. For laboratory samples immerged at different temperatures, the seawater was continuously filtered and renewed and this procedure probably limited the bacterial action which degrades the PHBV at sea. These results highlighted that degradation at lower temperature took place preferentially through an enzymatic process via a surface erosion mechanism, confirmed by the roughness evolution and the SEM images.

However, the study of the temperature influence revealed another degradation mechanism. Despite the higher crystallinity of PHBV, chain cleavage activated by the temperature (especially at 40°C) occurred caused by hydrolysis of the ester bonds. Mechanical and thermal properties confirmed this behavior.

Finally, the goal of this work was to establish a baseline for lifetime prediction of PHBV in a marine environment. In the methodology usually employed with accelerated thermal ageing, some assumptions must be respected. This study revealed the presence of two degradation mechanisms occurring in parallel (enzymatic degradation and chain scission) but the kind of degradation is dependent on the temperature and medium. According to the marine ageing conditions, one or other of these mechanisms is predominant. Use of prediction tools such as Arrhenius relationships is questionable if the ageing conditions in terms of temperature and presence of bacteria are not precisely defined. However, the activation energy is constant over the temperature range studied validating one of the assumptions for the use of prediction tools. Accelerated tests in distilled water avoiding the microorganism attack and only studying the water effect on PHBV samples may help to clarify the degradation kinetics. Further work to explore this is in progress.

Acknowledgements

The authors thank the SERPBIO association for financial support. The authors are also pleased to express their grateful acknowledgements to Anthony Magueresse, Dr. Mickaël Castro, Dr. Jean-Luc Audic and Dr. Patrick Loulergue for their help in the experimental work.

References

[1] Keshavarz T, Roy I. Polyhydroxyalkanoates: bioplastics with a green agenda. Curr Opin Microbiol 2010;13:321–6.
[2] Iles A, Martin AN. Expanding bioplastics production: sustainable business innovation in the chemical industry. J Clean Prod 2013;45:38–49.
[3] Galgani F, Jaunet S, Campillo A, Guenegen X, His E. Distribution and abundance of debris on the continental shelf of the north-western Mediterranean Sea. Mar Pollut Bull 1995;30:713–7.
[4] Pichel WG, Churnside JH, Veenstra TS, Foley DG, Friedman KS, Brainard RE. Marine debris collects within the North Pacific Subtropical Convergence Zone. Mar Pollut Bull 2007;54:1207–11.
[5] Barnes DKA, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc B Biol Sci 2009;364:1985–98.
[6] Boerger CM, Lattin GL, Moore SL, Moore CJ. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar Pollut Bull 2010;60:2275–8.
[7] Sudesh K, Abe H, Doi Y. Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. Prog Polym Sci 2000;25:1503–55.

[8] Doi Y, Kasuya K, Abe H, Koyama N, Shin-ichi I, Koichi T, et al. Evaluation of biodegradabilities of biosynthetic and chemosynthetic polyesters in river water. Polym Degrad Stab 1996;51:281–6.
[9] Kim S, Dale B. Life Cycle Assessment Study of Biopolymers (Polyhydroxyalkanoates) - Derived from No-Tilled Corn (11 pp). Int J Life Cycle Assess 2005;10:200–10.
[10] Harding KG, Dennis JS, von Blottnitz H, Harrison STL. Environmental analysis of plastic production processes: Comparing petroleum-based polypropylene and polyethylene with biologically-based poly-β-hydroxybutyric acid using life cycle analysis. J Biotechnol 2007;130:57–66.
[11] Holmes PA. Biologically Produced (R)-3-Hydroxy- Alkanoate Polymers and Copolymers. In: Bassett DC, editor. Dev. Cryst. Polym., Springer Netherlands; 1988, p. 1–65.
[12] Ohura T, Aoyagi Y, Takagi K, Yoshida Y, Kasuya K, Doi Y. Biodegradation of poly(3-hydroxyalkanoic acids) fibers and isolation of poly(3-hydroxybutyric acid)-degrading microorganisms under aquatic environments. Polym Degrad Stab 1999;63:23–9.
[13] Bruzaud S, Bourmaud A. Thermal degradation and (nano)mechanical behavior of layered silicate reinforced poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanocomposites. Polym Test 2007;26:652–9.
[14] Imam SH, Gordon SH, Shogren RL, Tosteson TR, Govind NS, Greene RV. Degradation of Starch–Poly(β-Hydroxybutyrate-Co-β-Hydroxyvalerate) Bioplastic in Tropical Coastal Waters. Appl Environ Microbiol 1999;65:431–7.
[15] Leathers TD, Govind NS, Greene RV. Biodegradation of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by a Tropical Marine Bacterium, Pseudoalteromonas sp. NRRL B-30083. J Polym Environ 2000;8:119–24.
[16] Sridewi N, Bhubalan K, Sudesh K. Degradation of commercially important polyhydroxyalkanoates in tropical mangrove ecosystem. Polym Degrad Stab 2006;91:2931–40.
[17] Volova TG, Boyandin AN, Vasiliev AD, Karpov VA, Prudnikova SV, Mishukova OV, et al. Biodegradation of polyhydroxyalkanoates (PHAs) in tropical coastal waters and identification of PHA-degrading bacteria. Polym Degrad Stab 2010;95:2350–9.
[18] Kasuya K, Takagi K, Ishiwatari S, Yoshida Y, Doi Y. Biodegradabilities of various aliphatic polyesters in natural waters. Polym Degrad Stab 1998;59:327–32.
[19] Tsuji H, Suzuyoshi K. Environmental degradation of biodegradable polyesters 1. Poly(ε-caprolactone), poly[(R)-3-hydroxybutyrate], and poly(L-lactide) films in controlled static seawater. Polym Degrad Stab 2002;75:347–55.
[20] Tsuji H, Suzuyoshi K. Environmental degradation of biodegradable polyesters 2. Poly(ε-caprolactone), poly[(R)-3-hydroxybutyrate], and poly(L-lactide) films in natural dynamic seawater. Polym Degrad Stab 2002;75:357–65.
[21] Rutkowska M, Krasowska K, Heimowska A, Adamus G, Sobota M, Musioł M, et al. Environmental Degradation of Blends of Atactic Poly[(R,S)-3-hydroxybutyrate] with Natural PHBV in Baltic Sea Water and Compost with Activated Sludge. J Polym Environ 2008;16:183–91.
[22] Muhamad II, Lee KJ, Mohd. Noor MA. Comparing the degradation of Poly-B-(hydroxybutyrate), poly-B-(hydroxbutyrate-co-valerate)(PHBV) and PHBV/cellulose tricate blend. Malays Polym J 2006;1:39–46.
[23] Mukai K, Yamada K, Doi Y. Efficient hydrolysis of polyhydroxyalkanoates by Pseudomonas stutzeri YM1414 isolated from lake water. Polym Degrad Stab 1994;43:319–27.
[24] Weng Y-X, Wang Y, Wang X-L, Wang Y-Z. Biodegradation behavior of PHBV films in a pilot-scale composting condition. Polym Test 2010;29:579–87.
[25] Corre Y-M, Bruzaud S, Audic J-L, Grohens Y. Morphology and functional properties of commercial polyhydroxyalkanoates: A comprehensive and comparative study. Polym Test 2012;31:226–35.
[26] Arbelaiz A, Fernández B, Ramos JA, Retegi A, Llano-Ponte R, Mondragon I. Mechanical properties of short flax fibre bundle/polypropylene composites: Influence of matrix/fibre modification, fibre content, water uptake and recycling. Compos Sci Technol 2005;65:1582–92.
[27] Barham PJ, Keller A, Otun EL, Holmes PA. Crystallization and morphology of a bacterial thermoplastic: poly-3-hydroxybutyrate. J Mater Sci 1984;19:2781–94.

[28] Avella M, Rota GL, Martuscelli E, Raimo M, Sadocco P, Elegir G, et al. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and wheat straw fibre composites: thermal, mechanical properties and biodegradation behaviour. J Mater Sci 2000;35:829–36.
[29] Tang CY, Chen DZ, Yue TM, Chan KC, Tsui CP, Yu PHF. Water absorption and solubility of PHBHV/HA nanocomposites. Compos Sci Technol 2008;68:1927–34.
[30] Le Duigou A, Davies P, Baley C. Seawater ageing of flax/poly(lactic acid) biocomposites. Polym Degrad Stab 2009;94:1151–62.
[31] Huang Y, Zhang C, Pan Y, Zhou Y, Jiang L, Dan Y. Effect of NR on the hydrolytic degradation of PLA. Polym Degrad Stab 2013;98:943–50.
[32] Burkersroda F von, Schedl L, Göpferich A. Why degradable polymers undergo surface erosion or bulk erosion. Biomaterials 2002;23:4221–31.
[33] Deroiné M, Le Duigou A, Corre Y-M, Le Gac P-Y, Davies P, Bruzaud S. Accelerated ageing of polylactide in aqueous environments: Comparative study between distilled water and seawater. Polym Degrad Stab 2014;DOI: 10.1016/j.polymdegradstab.2014.01.020.
[34] Taguchi S, Iwata T, Abe H, Doi Y. 9.09 - Poly(hydroxyalkanoate)s. In: Matyjaszewski K, Möller M, editors. Polym. Sci. Compr. Ref., Amsterdam: Elsevier; 2012, p. 157–82.
[35] Numata K, Abe H, Doi Y. Enzymatic processes for biodegradation of poly(hydroxyalkanoate)s crystals. Can J Chem 2008;86:471–83.
[36] Mukai K, Yamada K, Doi Y. Enzymatic degradation of poly(hydroxyalkanoates) by a marine bacterium. Polym Degrad Stab 1993;41:85–91.
[37] Sudesh K, Abe H. Practical Guide to Microbial Polyhydroxyalkanoates. iSmithers Rapra Publishing; 2010.
[38] Corrêa MCS, Rezende ML, Rosa DS, Agnelli JAM, Nascente PAP. Surface composition and morphology of poly(3-hydroxybutyrate) exposed to biodegradation. Polym Test 2008;27:447–52.
[39] Mergaert J, Webb A, Anderson C, Wouters A, Swings J. Microbial degradation of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in soils. Appl Environ Microbiol 1993;59:3233–8.
[40] Li S, McCarthy S. Further investigations on the hydrolytic degradation of poly (DL-lactide). Biomaterials 1999;20:35–44.

Table 1. Hydrochemical parameters of seawater.

	Temperature of water[a] (°C)	pH[a]	Salinity[b] (ppm)	Oxygen[b] ($mg.mL^{-1}$)
March 2012	10.9 ± 0.6	8.2	34.8	6.5
April	11.3 ± 0.3	7.9	34.6	7.4
May	13.5 ± 0.1	8.1	35	6.2
June	15 ± 0.1	8.0	34.9	6.7
July	18.4 ± 1.0	8.0	34.2	6.2
August	19.8 ± 0.8	8.0	34.7	6.3
September	17.2 ± 0.2	8.0	34.7	5.3
October	15.4 ± 1.0	8.5	35.3	5.6
November	12.0 ± 0.6	7.9	35.5	5.7
December	10.5 ± 0.8	8.1	34.7	6
January	9.0 ± 1.0	7.9	33.5	6.3
February	8.9 ± 0.9	8.1	33	6.6
March 2013	8.6 ± 0.9	8.1	34.5	6.8

[a] Personal data
[b] IFREMER buoy data

Table 2. Microbial enumeration contained in different seawater.

	Microbial counting ($CFU.mL^{-1}$)
Natural seawater	17000
Bath at 4°C	3400
Bath at 25°C	1600
Bath at 40°C	17000

Table 3. Experimental equilibrium water uptake for the different temperatures.

T (°C)	M_∞ (%)
4	0.22
13.1*	0.33
25	0.42
40	0.78

* Average value for natural ageing

Table 4. Fickian diffusion coefficients for PHBV specimens obtained by DVS.

T(°C)	$D.10^{-12}$ ($m^2.s^{-1}$)
20	1.9
30	3.6
40	8.0
50	28.1

Table 5. Evolution of PHBV mechanical properties before and after ageing in seawater at different temperatures.

T (°C)	Immersion condition	Immersion time (days)	Section used	E (MPa)		σ_b (MPa)		ε_b (%)	
	unaged		S_i*	4390 ± 102		35 ± 1.2		1.4 ± 0.1	
	natural ageing	360	S = f(t)	4687 ± 168	+ 7%	36.7 ± 0.6	+ 5%	1.1 ± 0.1	- 21%
			$S = S_i$	4545 ± 152	+ 4%	33.8 ± 0.9	- 3%		
4	seawater	360	S = f(t)	4544 ± 160	+ 4%	38.5 ± 0.2	+ 10%	1.5 ± 0.1	+ 7%
			$S = S_i$	4332 ± 175	- 2%	37.9 ± 0.3	+ 8%		
25	seawater	360	S = f(t)	4614 ± 102	+ 5%	35.9 ± 1.0	+ 3%	1.2 ± 0.1	- 14%
			$S = S_i$	4671 ± 128	+ 6%	35.7 ± 0.6	+ 2%		
40	seawater	360	S = f(t)	5531 ± 81	+ 26%	30.3 ± 1.0	- 14%	0.6 ± 0.1	- 58%
			$S = S_i$	5466 ± 75	+ 24%	27.9 ± 1.2	- 20%		

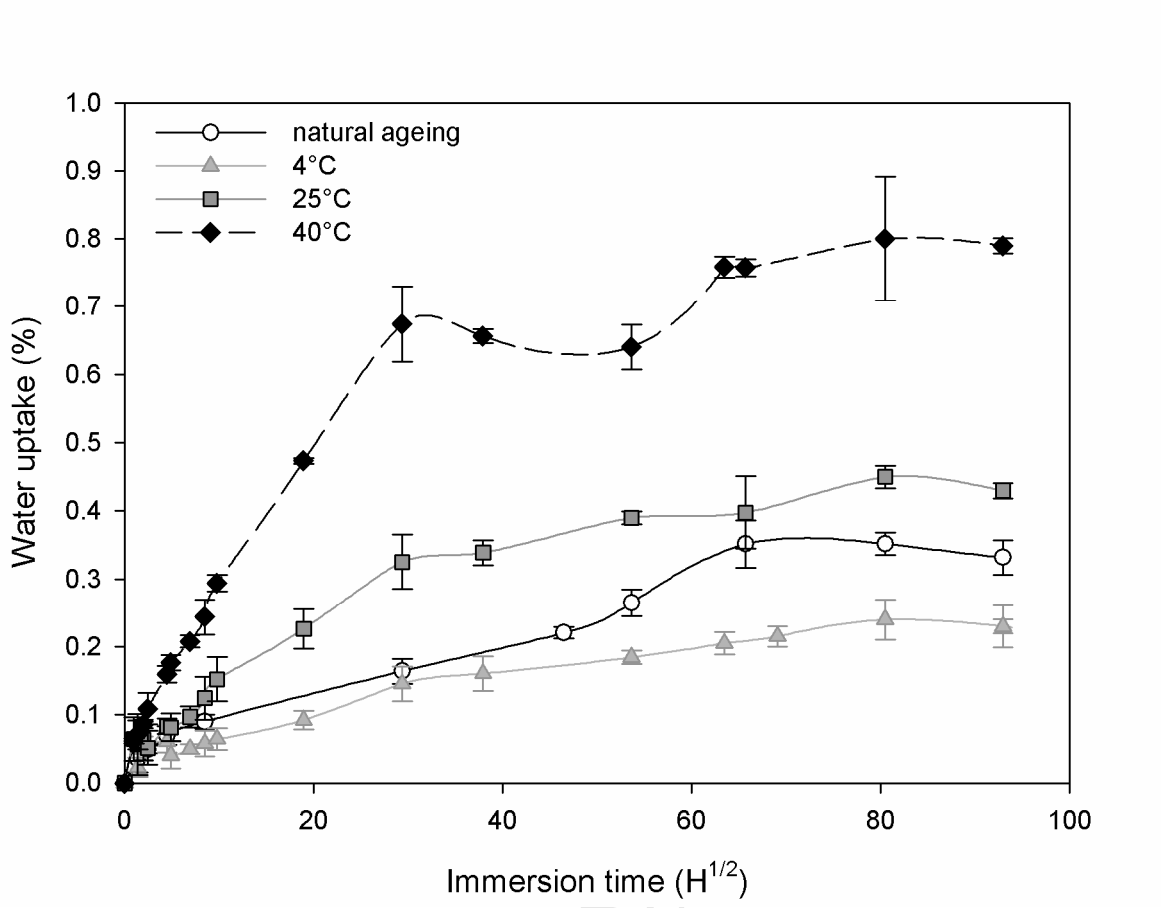
* S_i: initial section

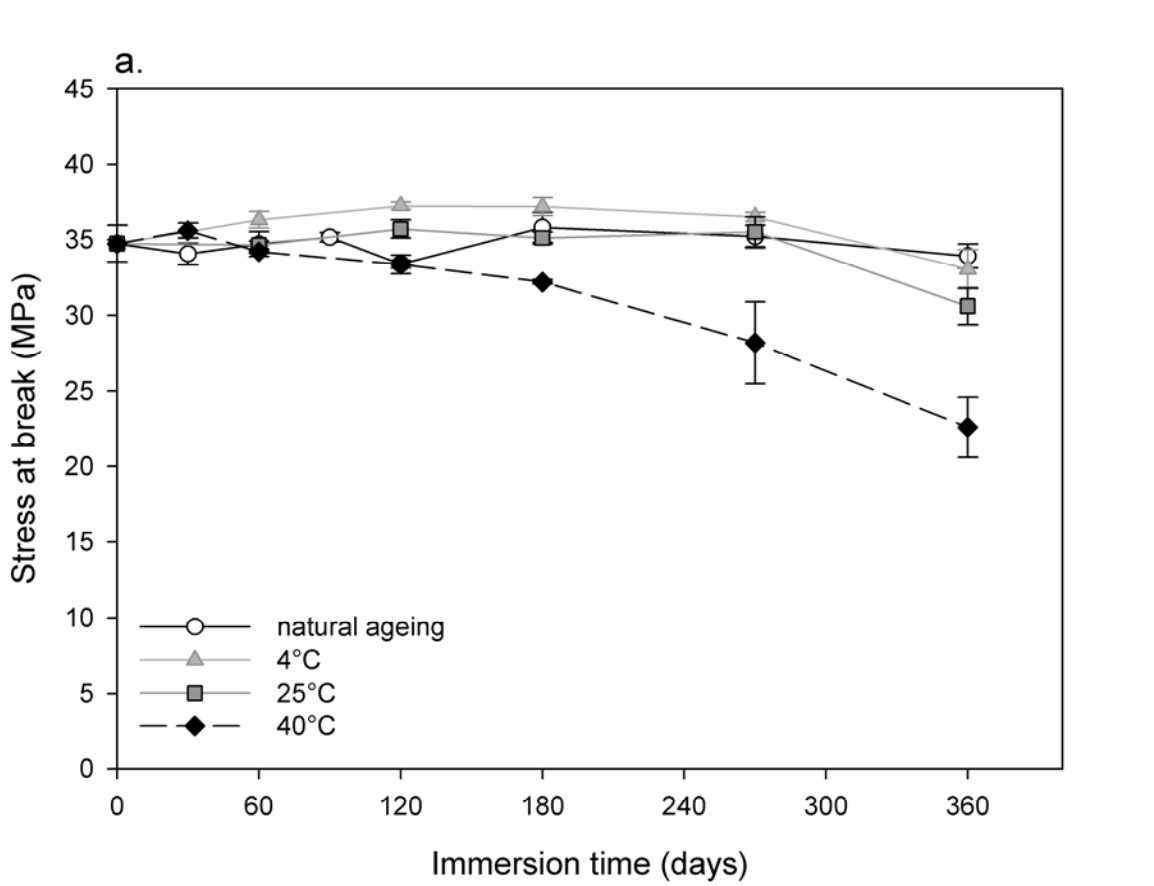
Table 6. Evolution of number-average molecular weight ($\overline{M}_n$), weight-average molecular weight ($\overline{M}_w$) and polydispersity index $\overline{M}_w/\overline{M}_n$ of PHBV samples aged 12 months in different seawater.

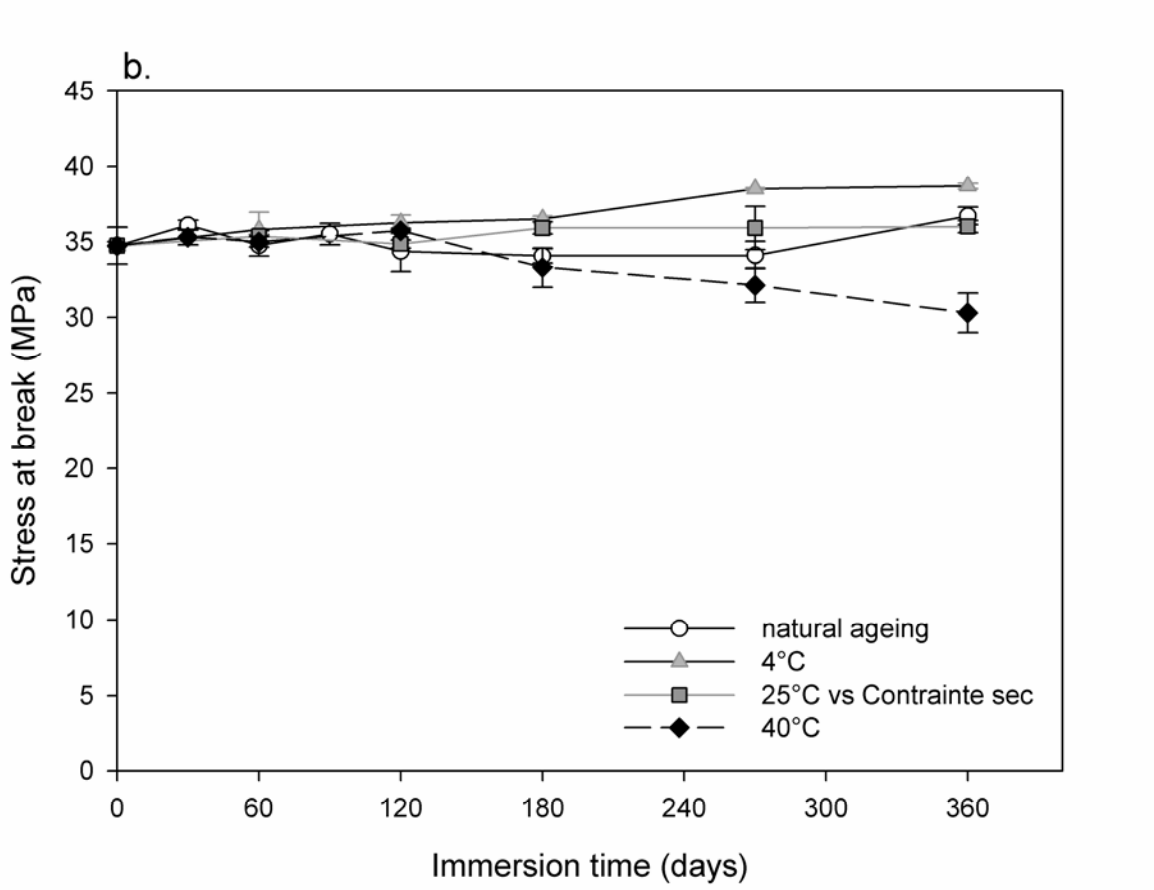
			$\overline{M}_n$ (g.mol^{-1})	$\overline{M}_w$ (g.mol^{-1})	$\overline{M}_w/\overline{M}_n$
	unaged	0	172600	398800	2.3
	natural ageing	360	145900	291000	2.0
4°C	seawater	360	172500	291800	1.7
25°C	seawater	360	164700	260300	1.6
40°C	seawater	360	82100	125300	1.5

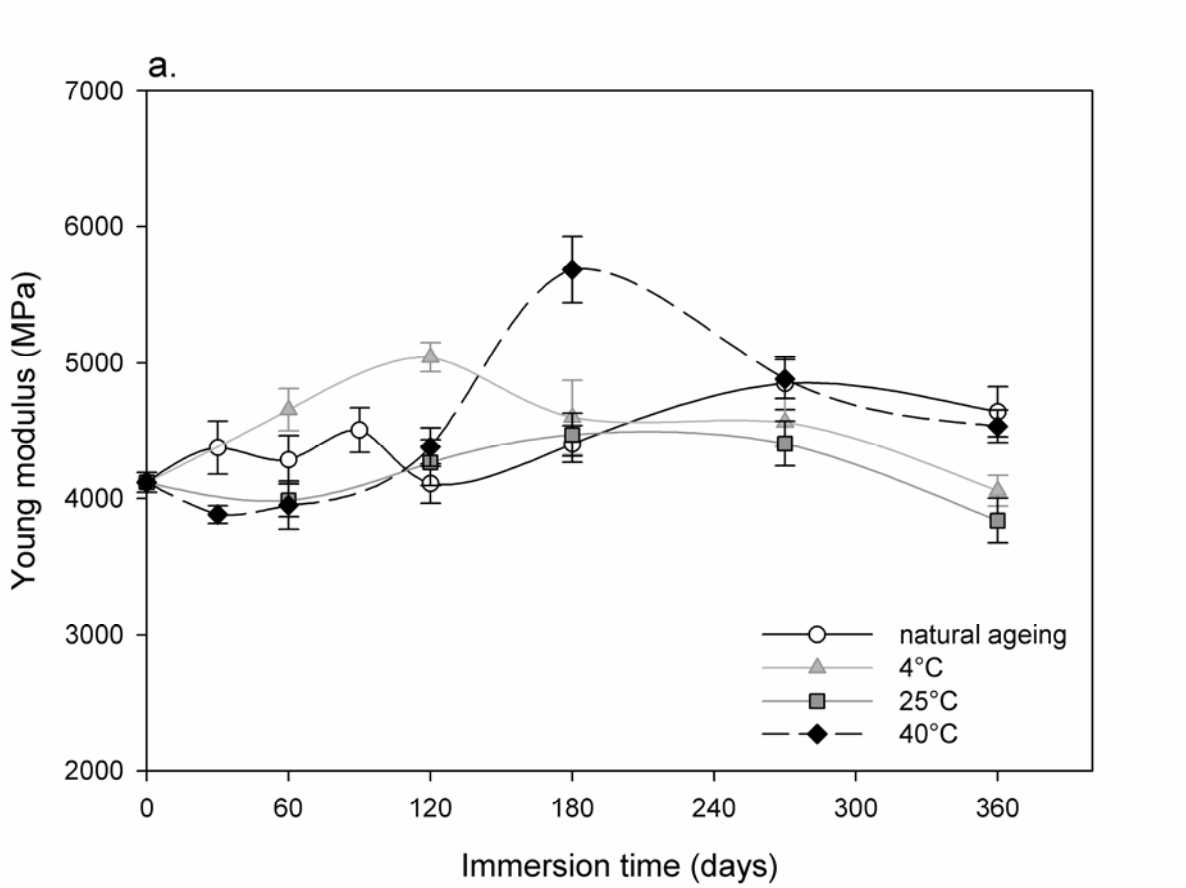
Table 7. Evolution of the thermal transitions of PHBV samples aged 12 months in seawater.

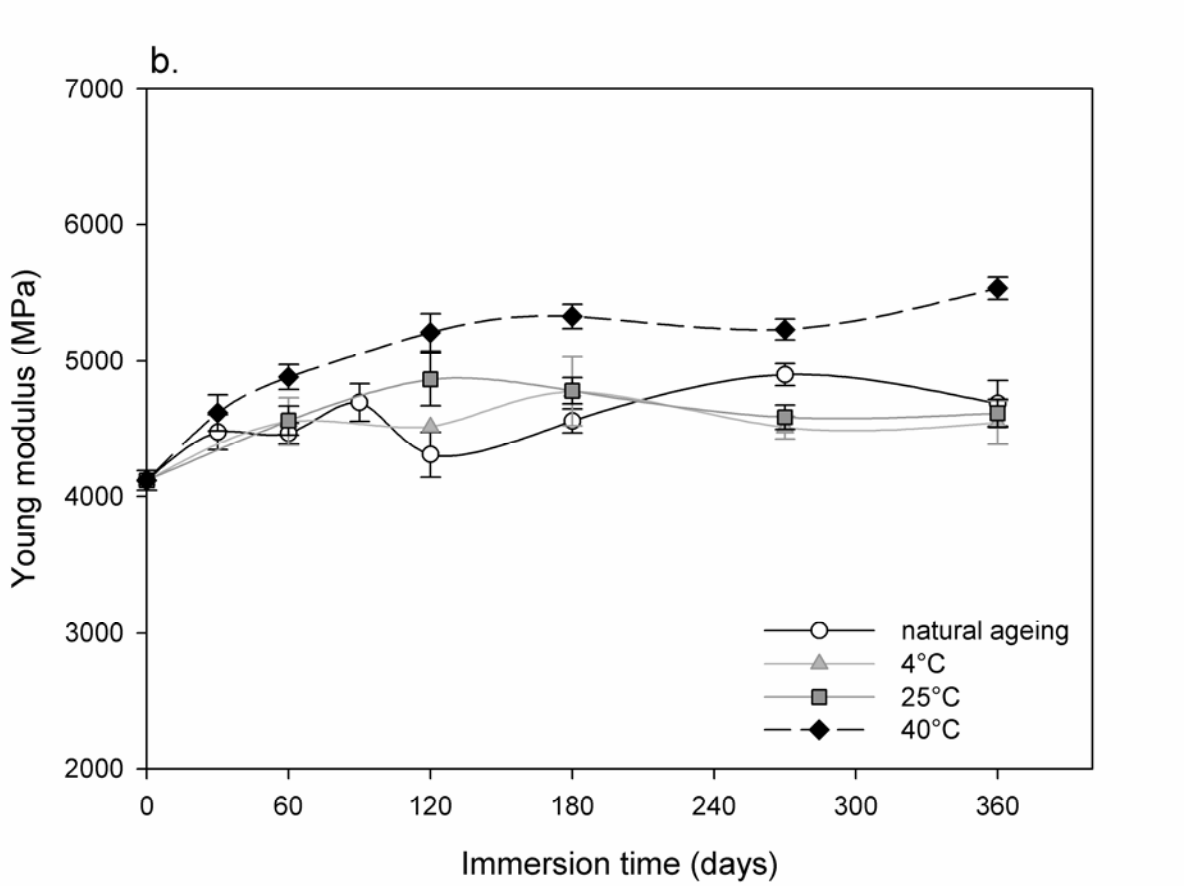
	1st heating			Cooling		2nd heating		
	T_m (°C)	ΔH_m (J.g^{-1})	χ_c (%)	$T_{c\,(°C)}$	ΔH_c (J.g^{-1})	T_m (°C)	ΔH_m (J.g^{-1})	χ_c (%)
Unaged	177	73	50	111	73	174	83	57
Natural ageing	179	80	55	109	69	171	90	62
4°C	178	68	47	111	77	171	86	59
25°C	176	70	48	109	75	171	86	59
40°C	173	88	60	110	86	167	98	67

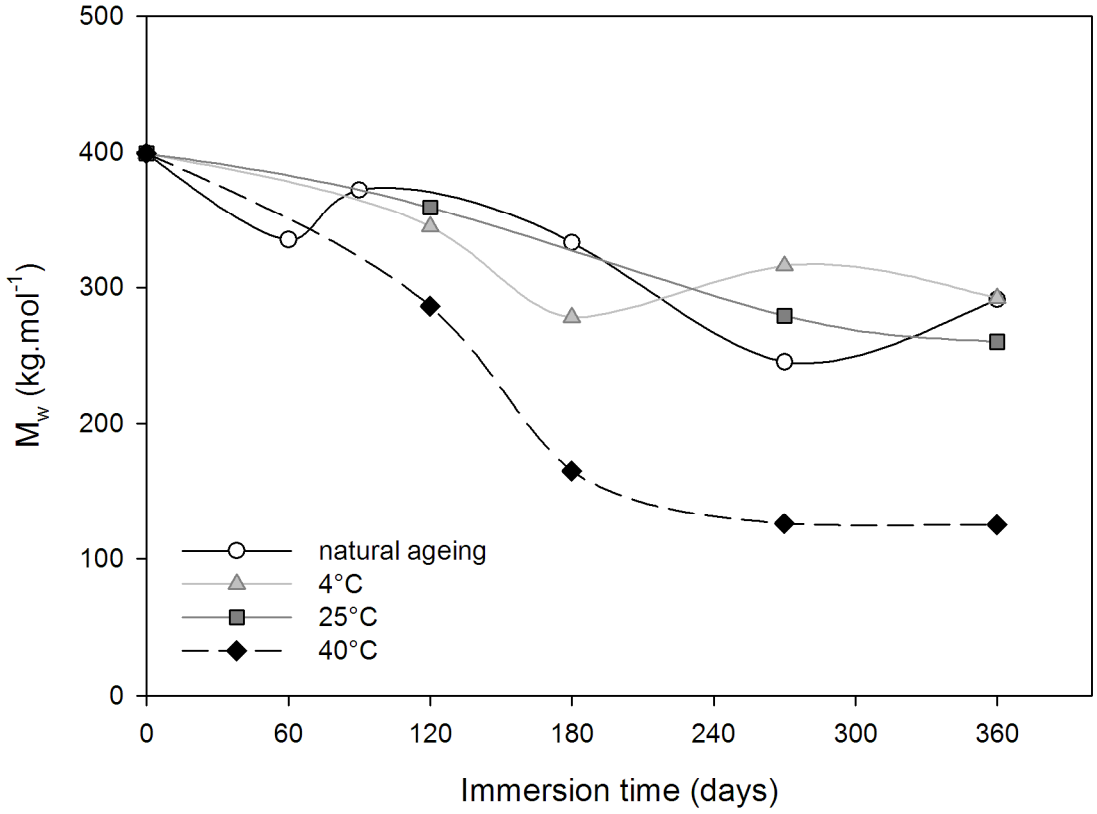


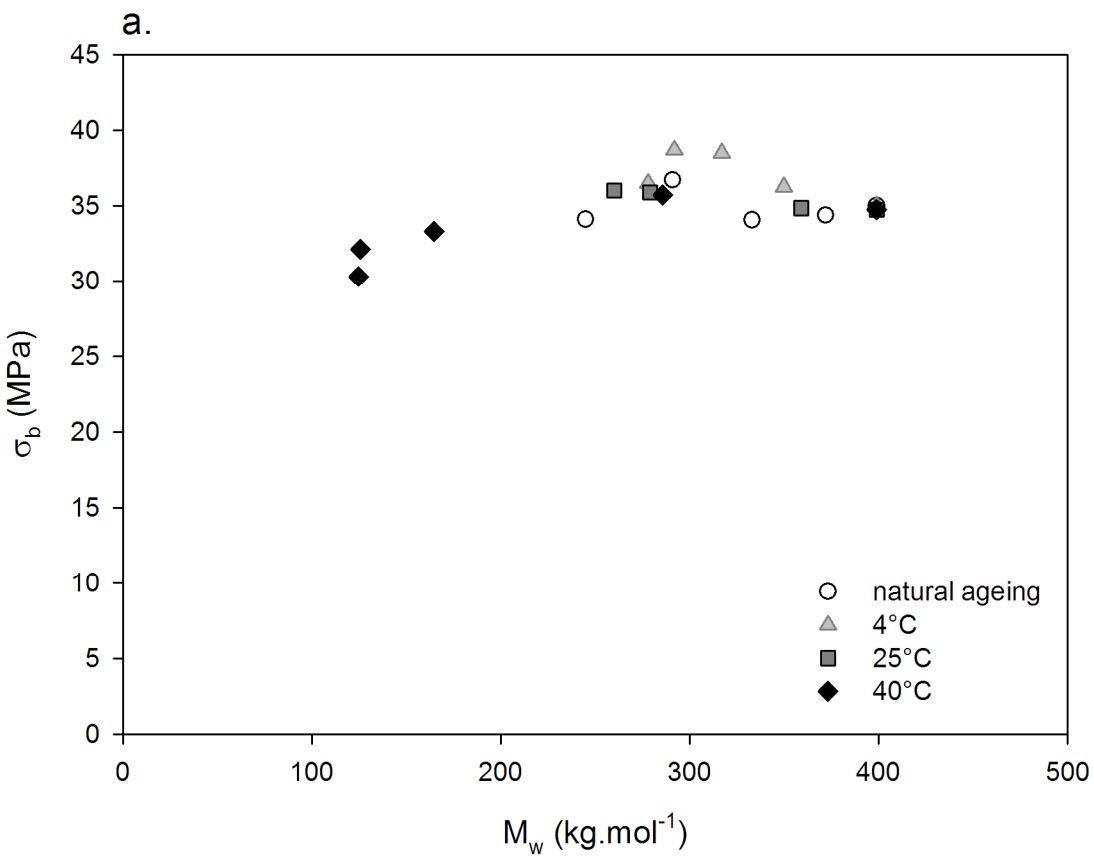


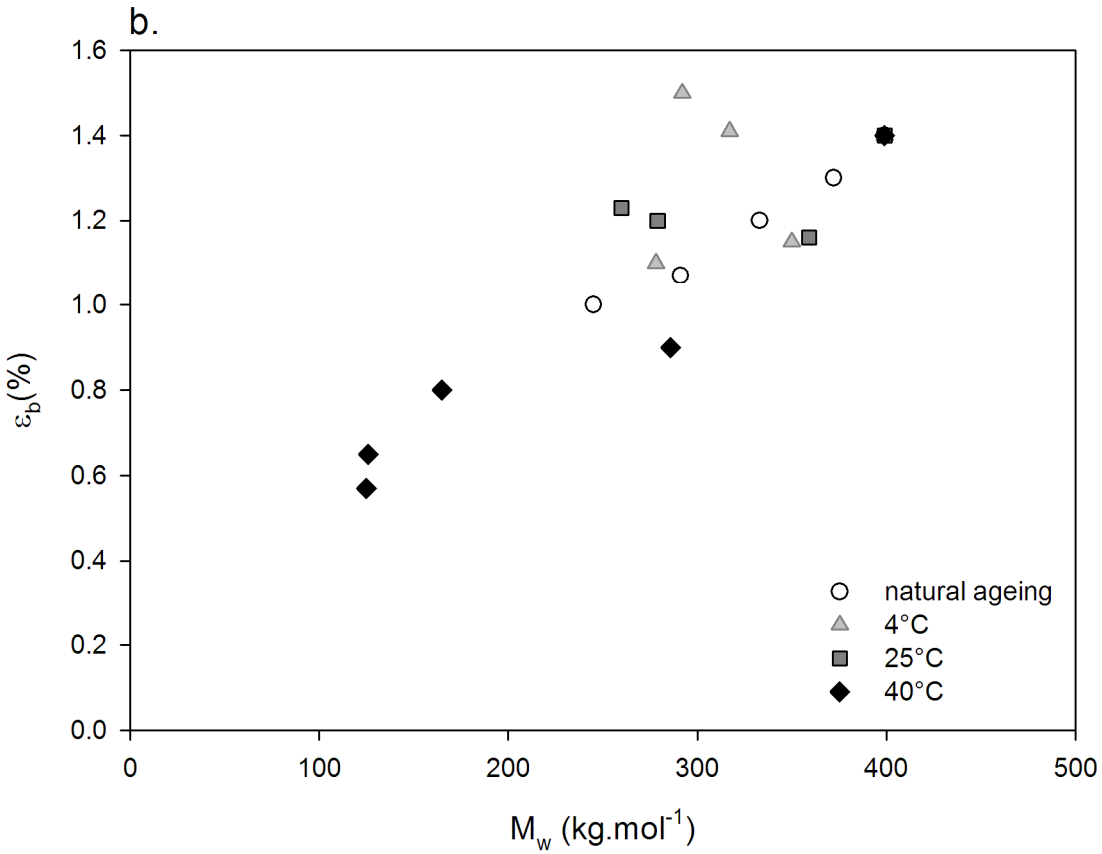


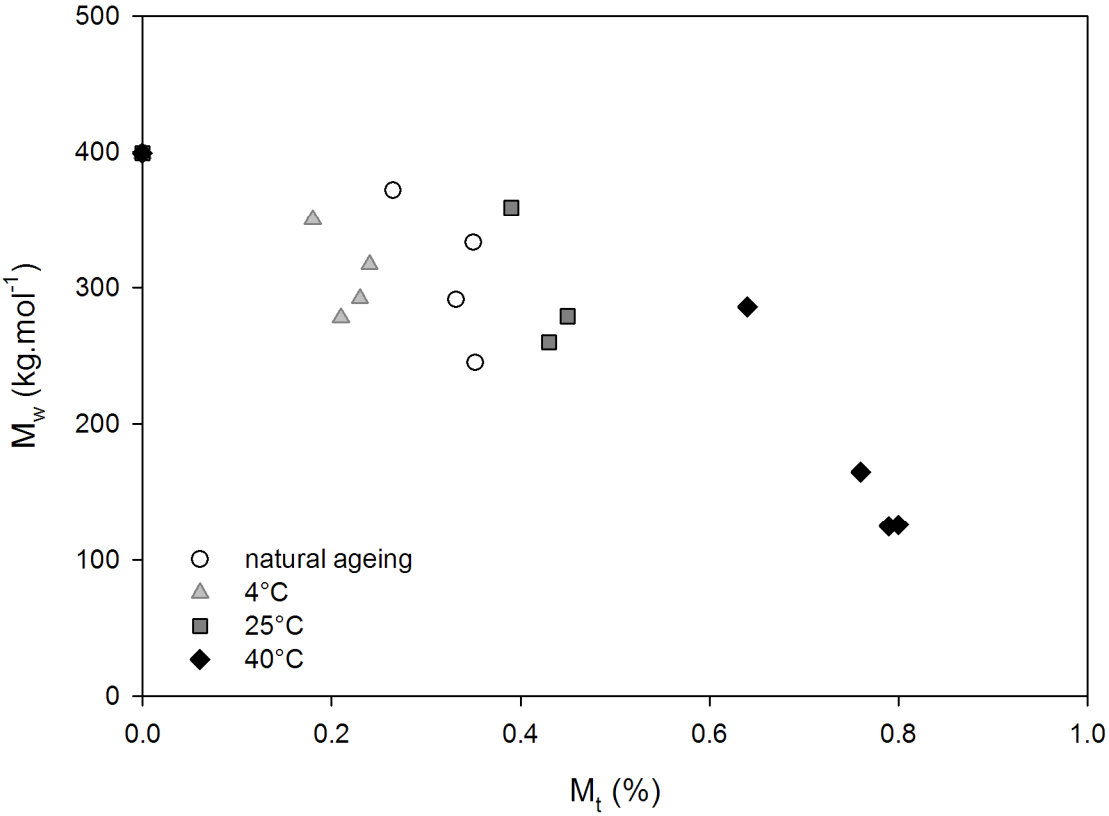


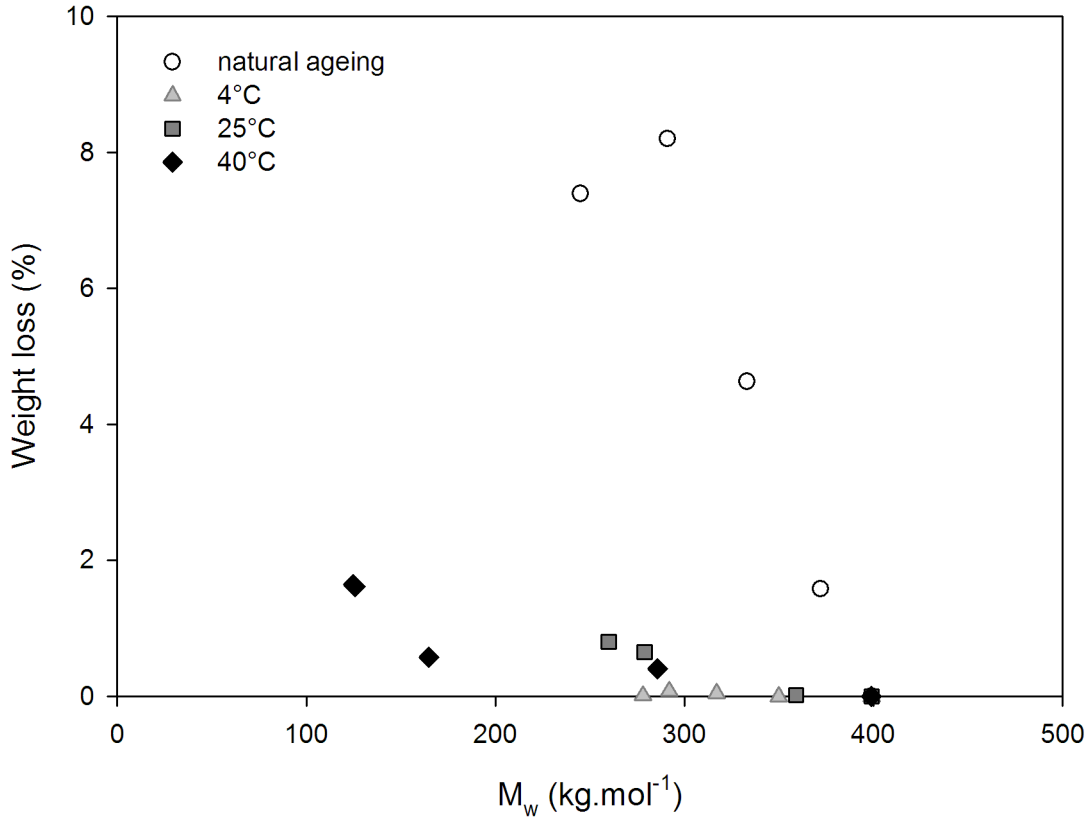


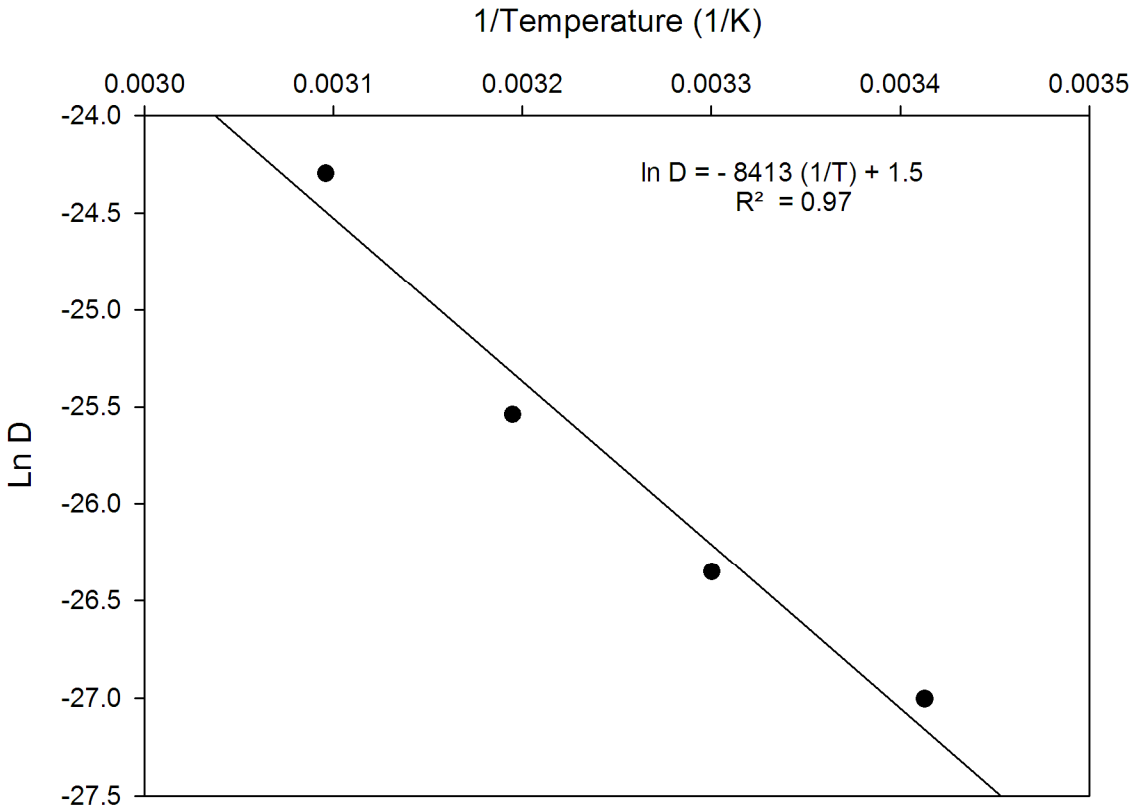


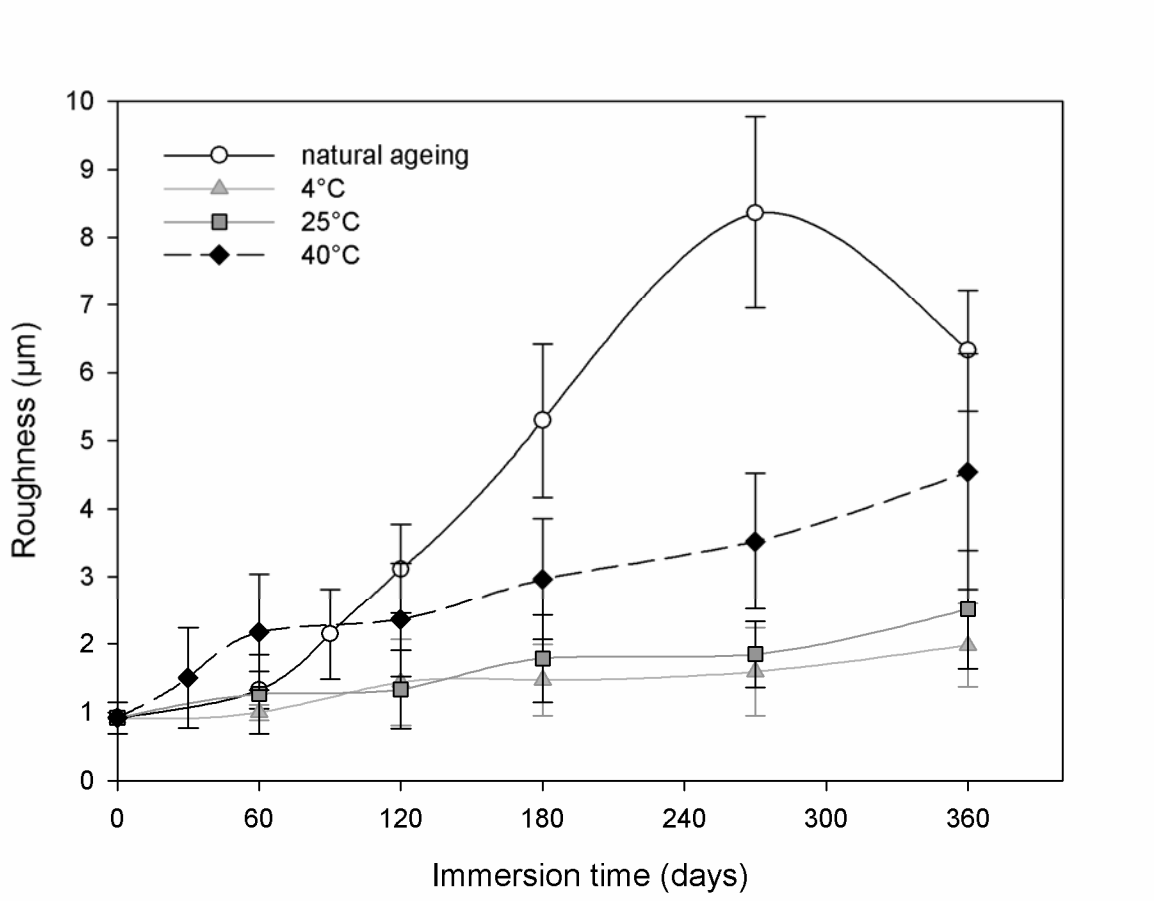


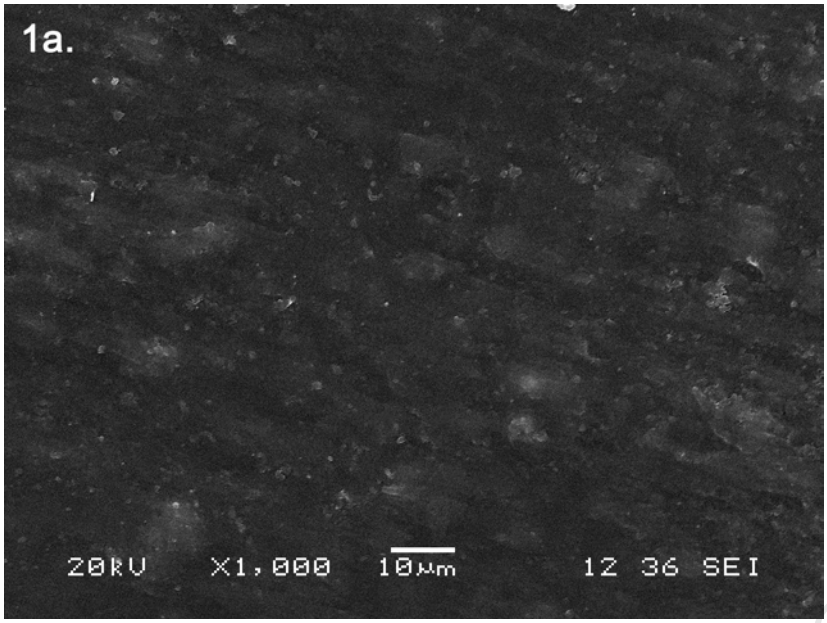


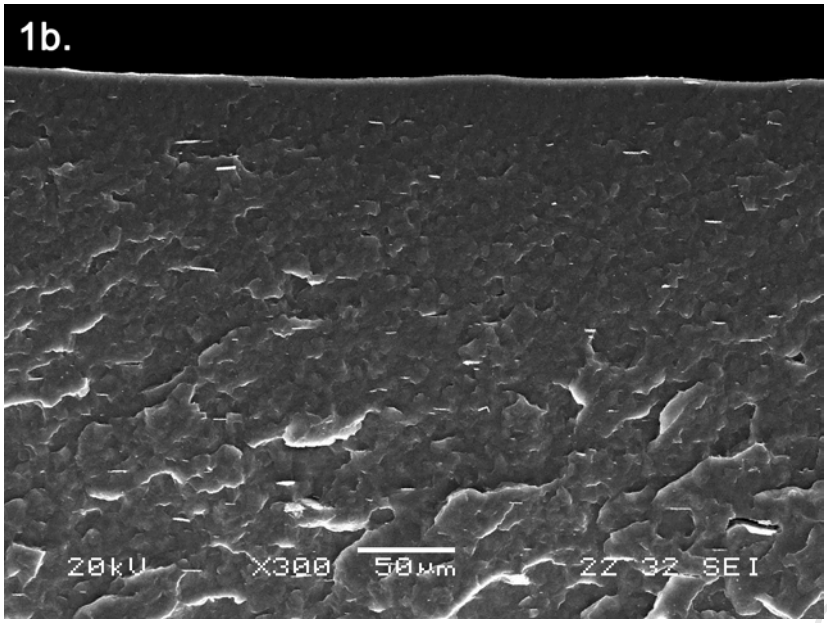


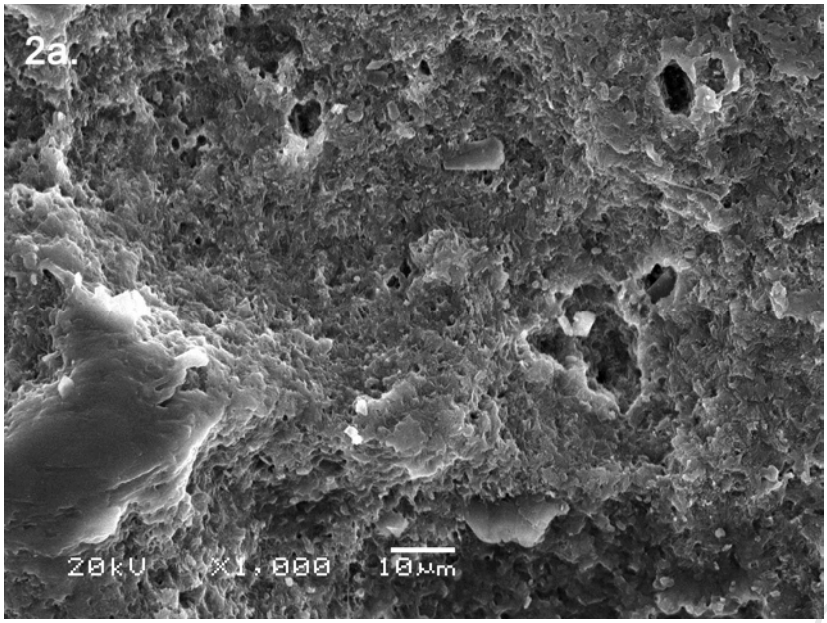


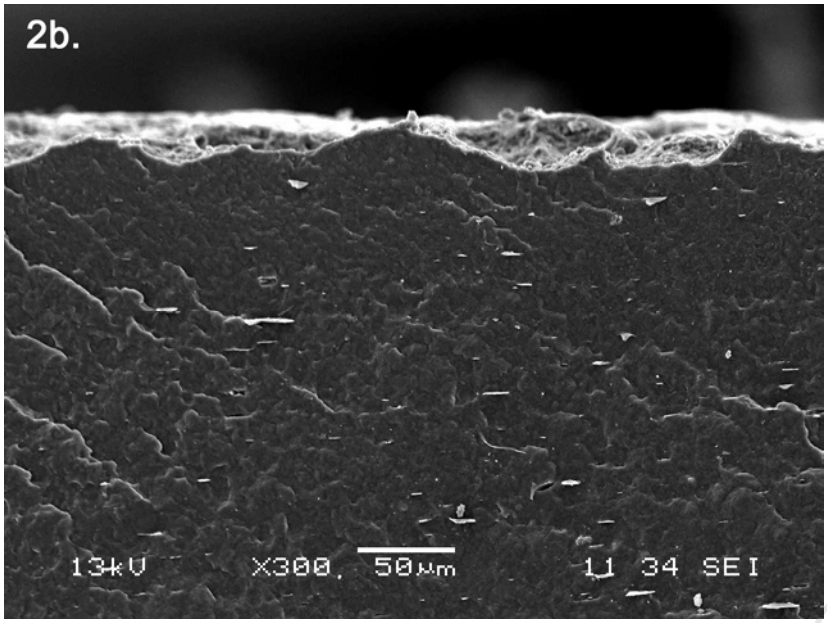


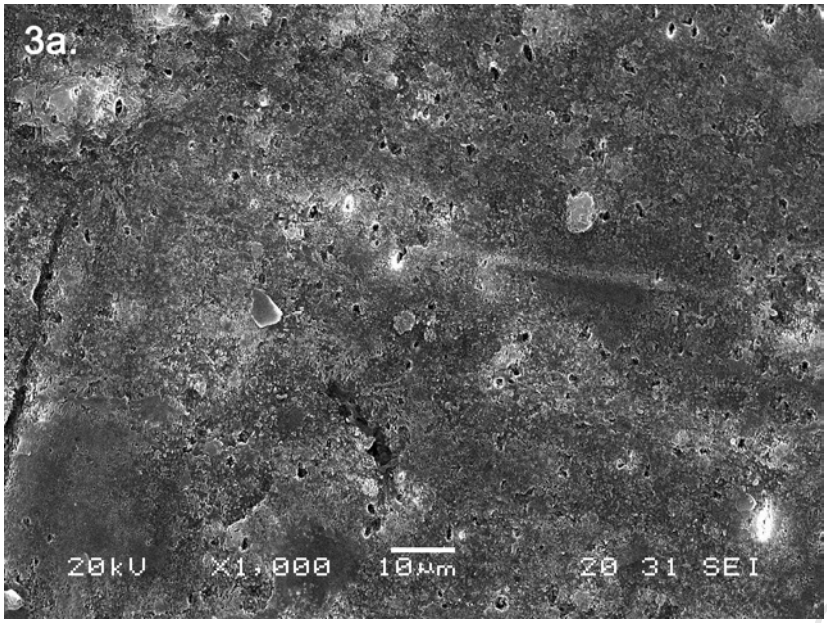


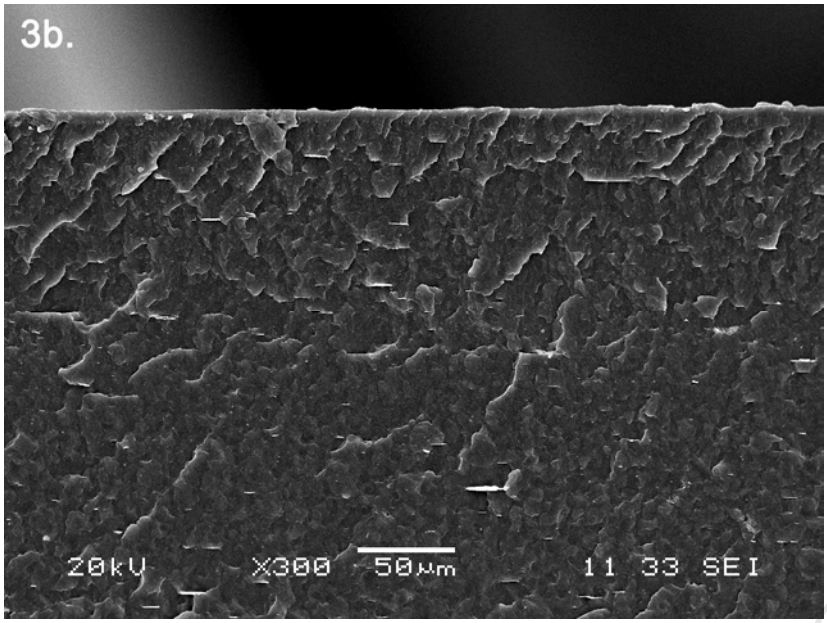


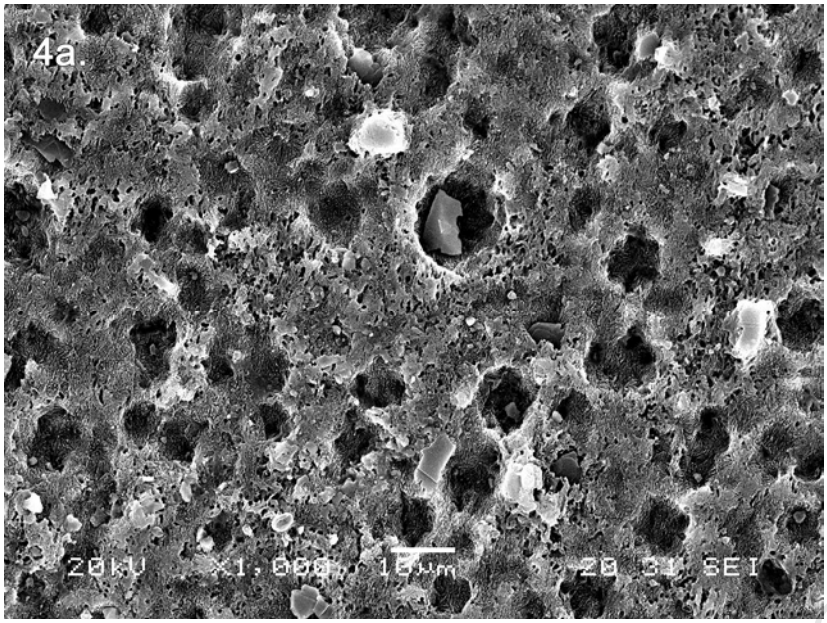


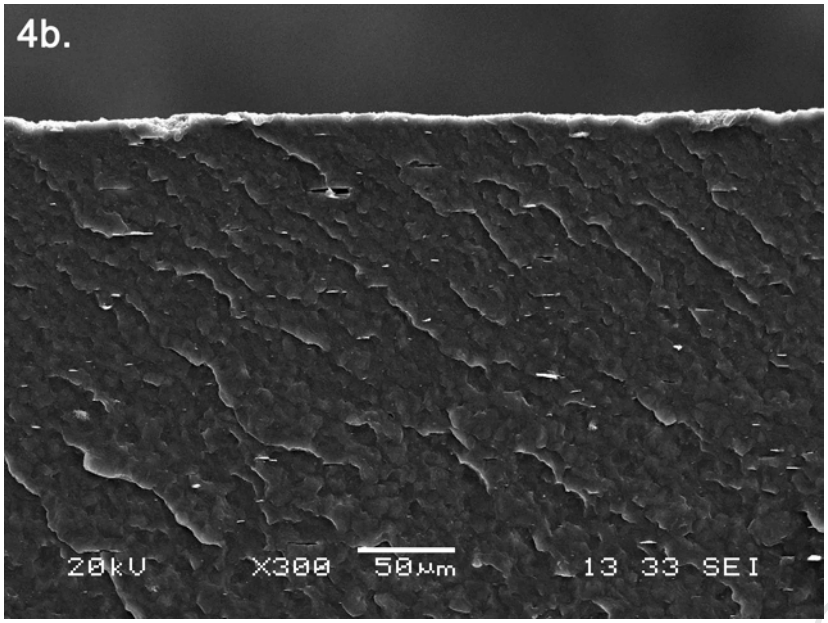


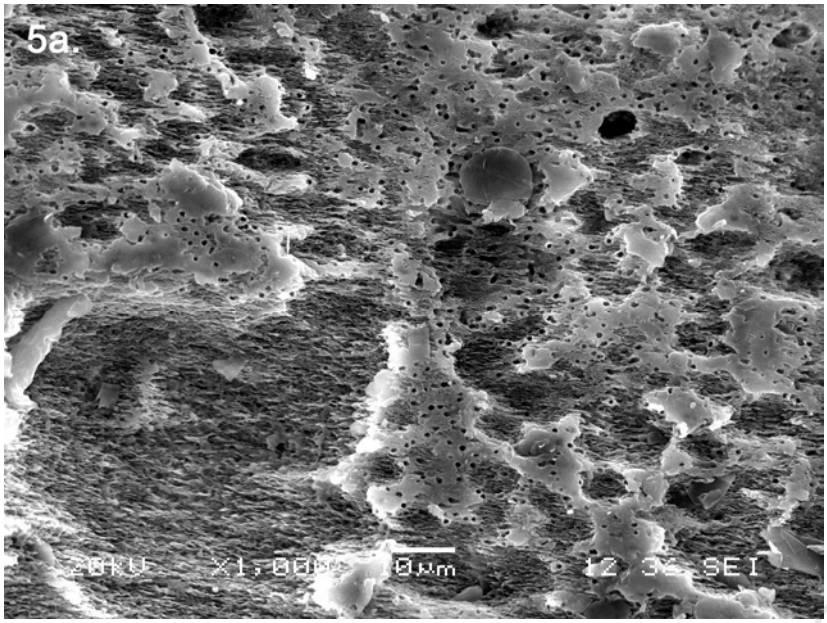


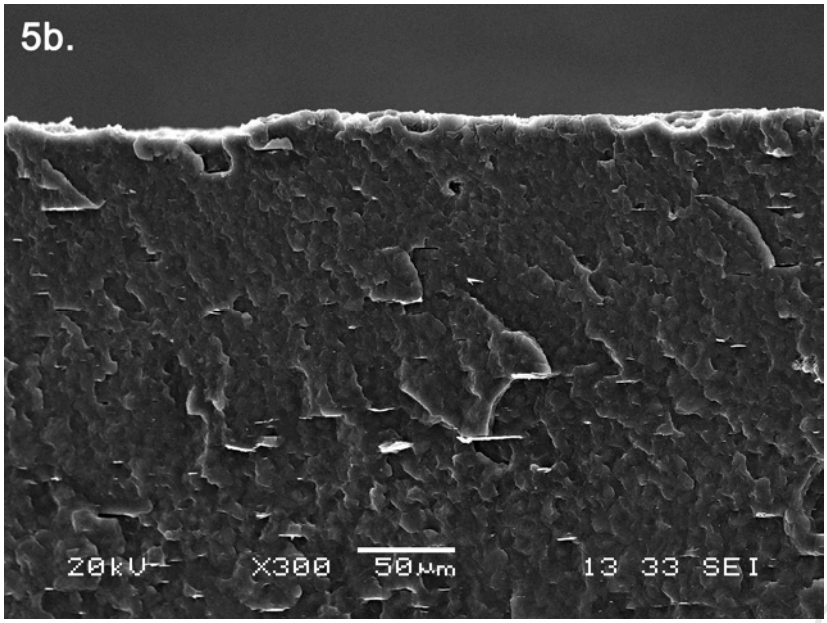


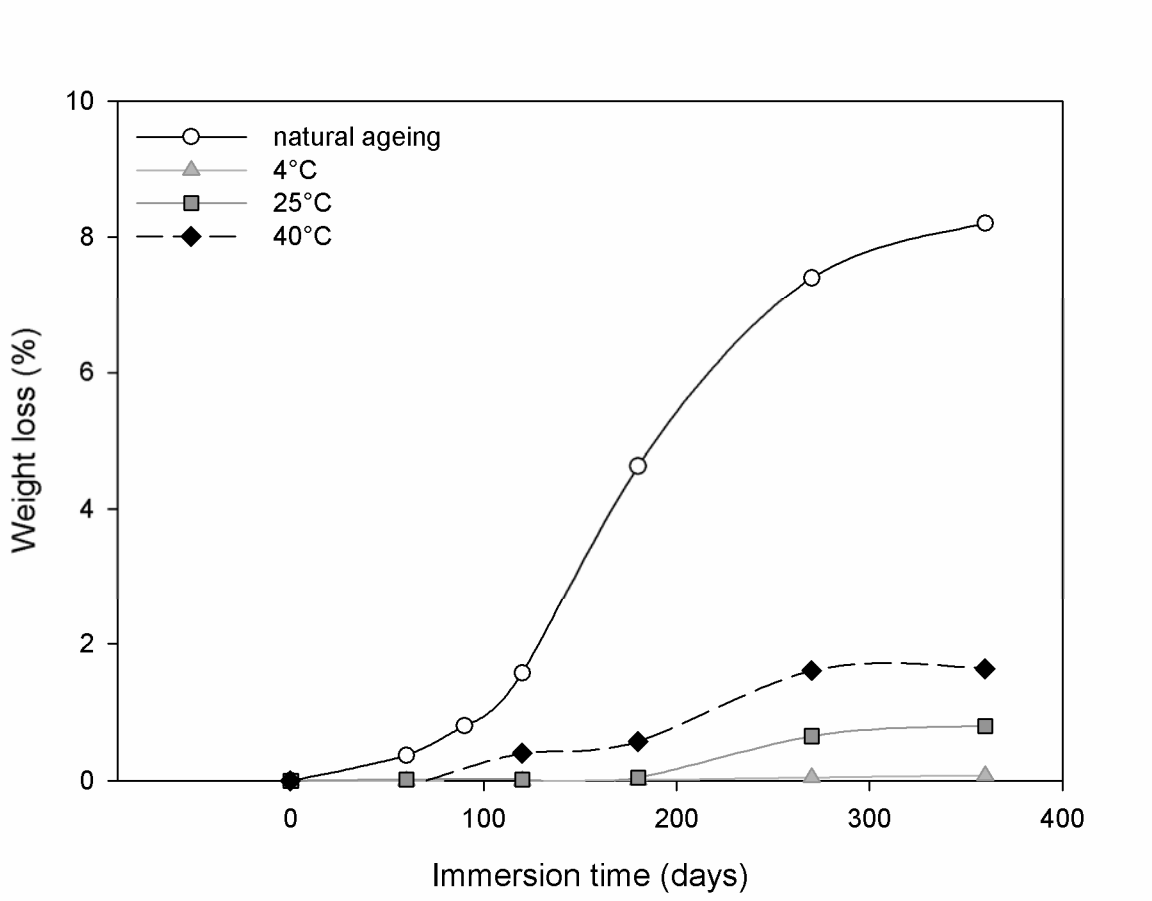


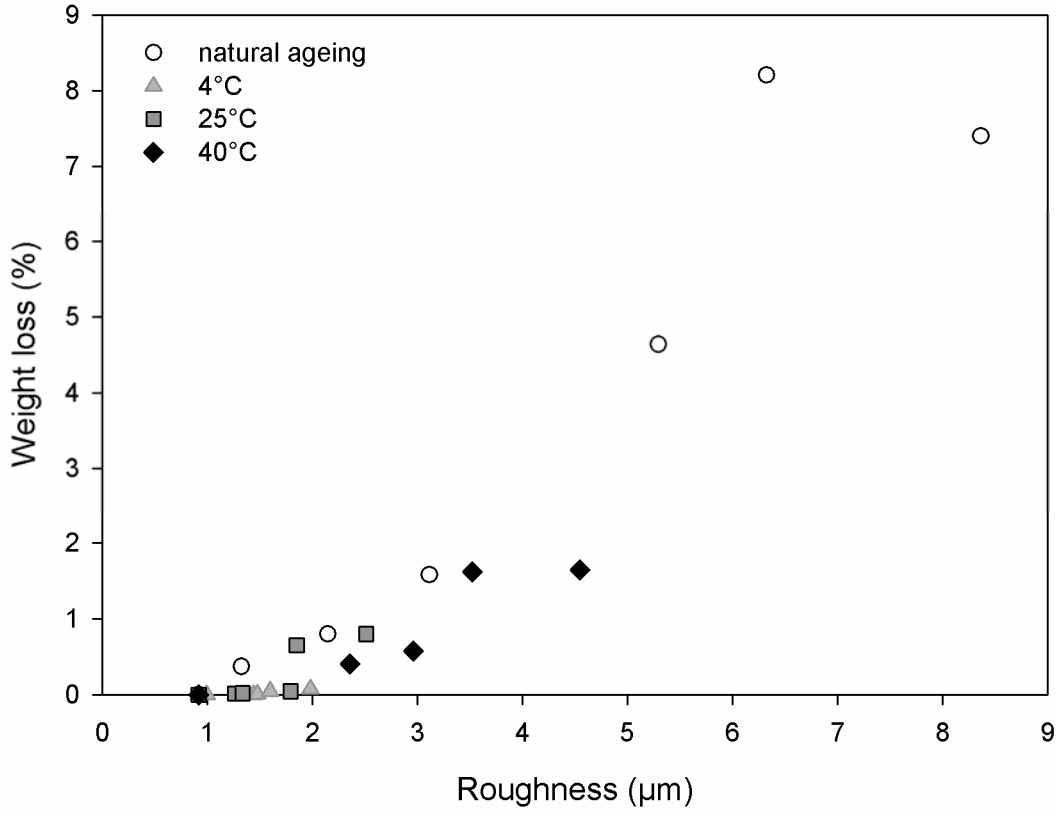








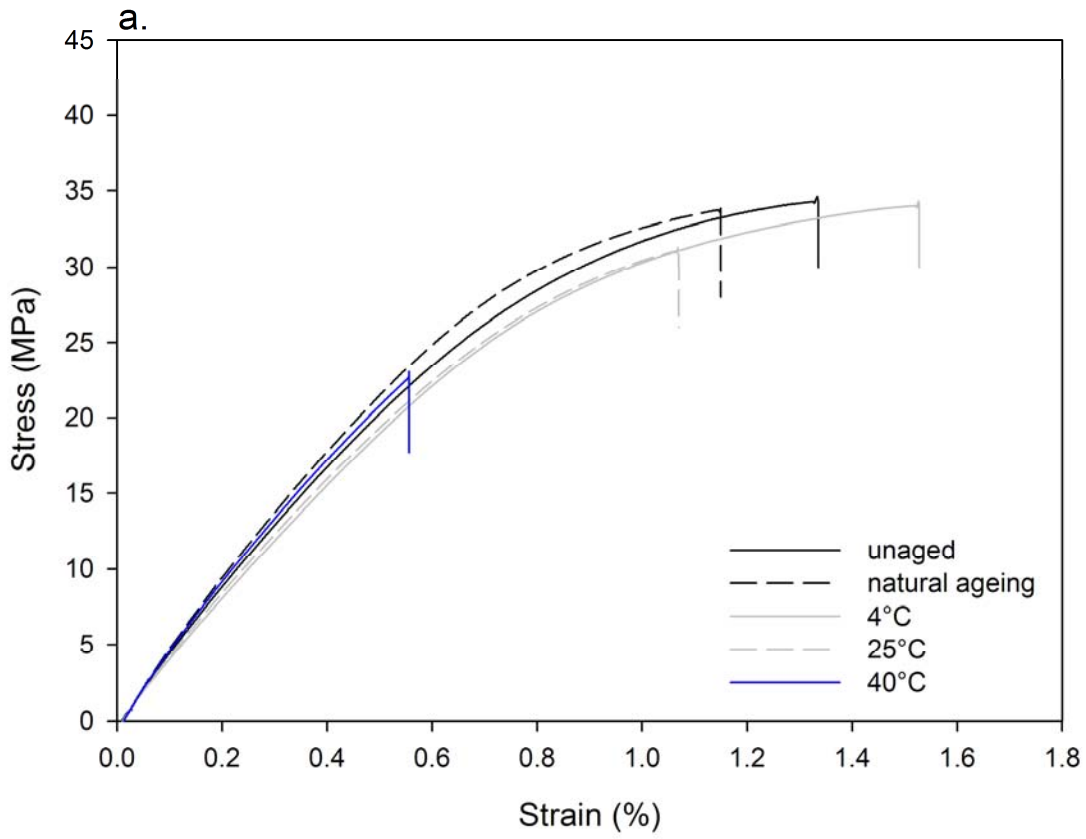


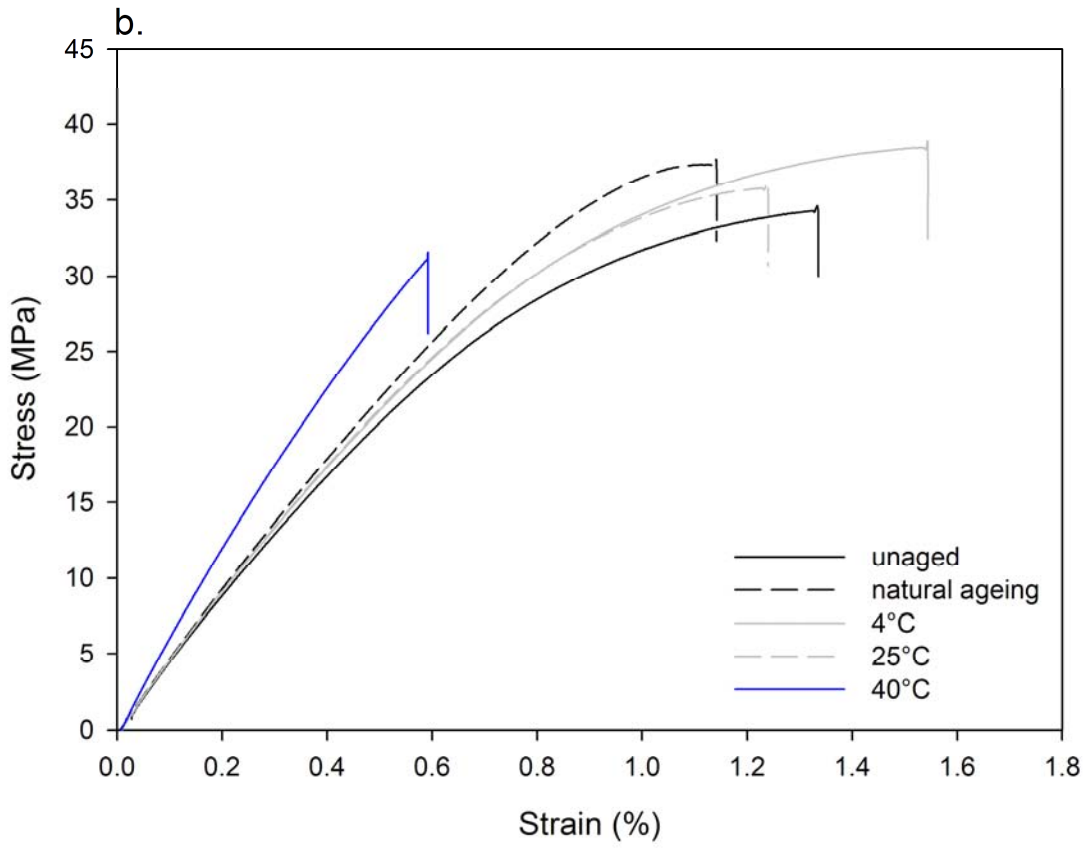


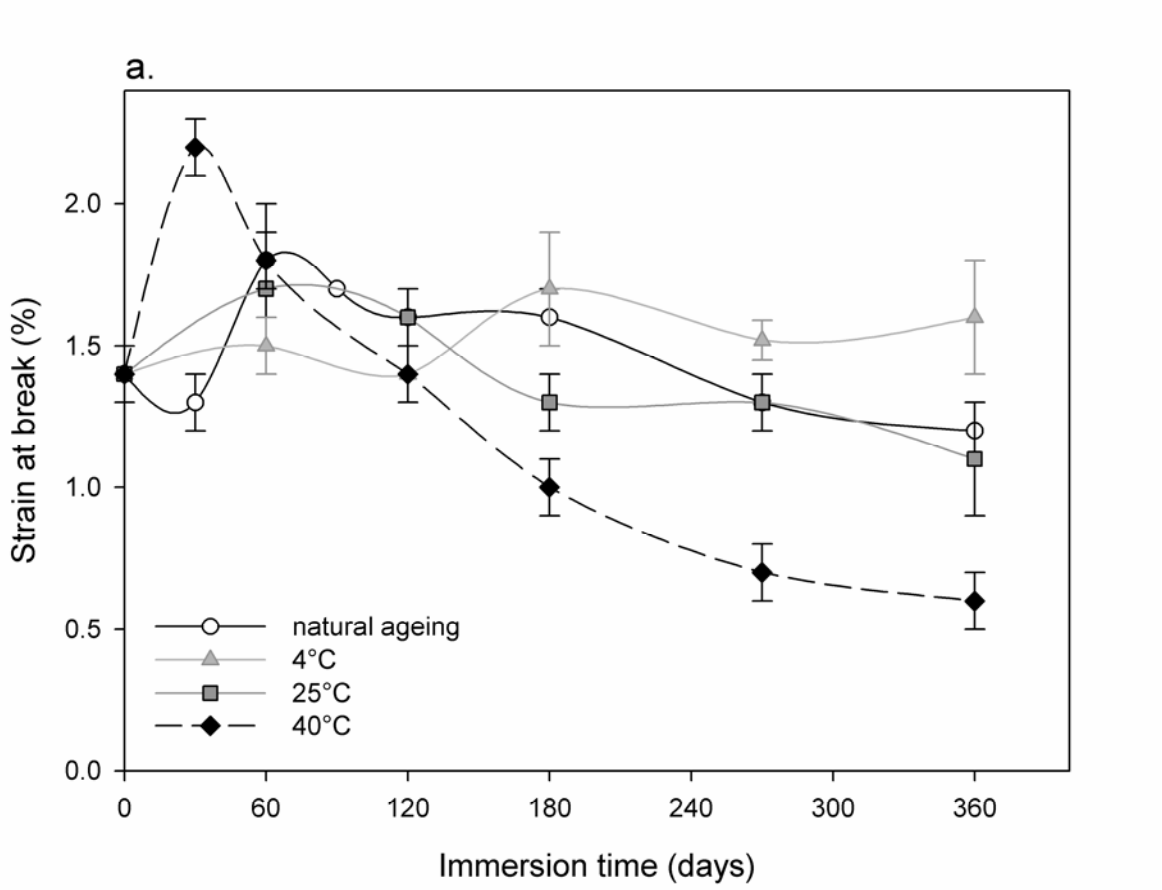
PHBV unaged

PHBV aged during 270 days

PHBV aged during 360 days







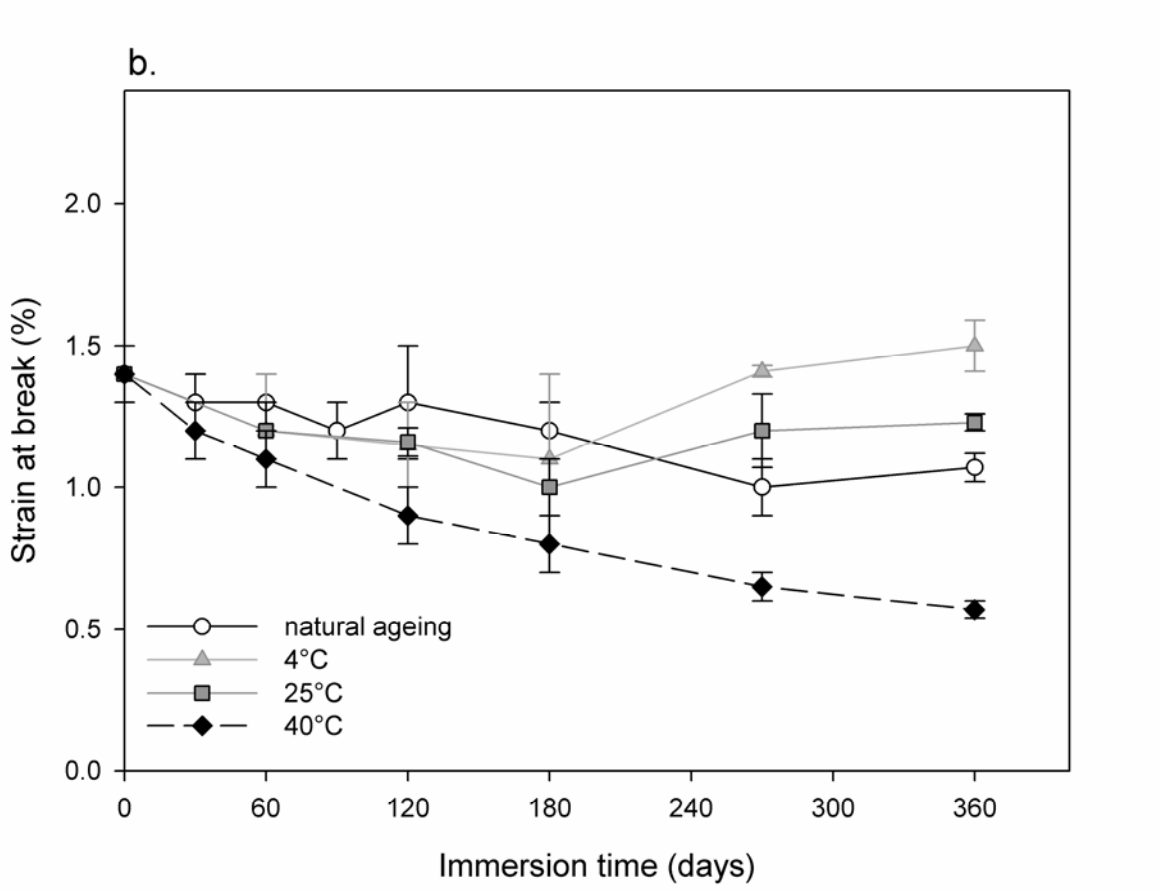


Fig. 1. Evolution of water uptake as function of the square root of immersion time at different temperatures in seawater.

Fig. 2. Identification of the diffusion coefficient of seawater in the PHBV for different ageing temperatures.

Fig. 3. Evolution of roughness of the PHBV for different ageing conditions.

Fig. 4. SEM micrographs of the surface (a) and the fracture (b) for unaged PHBV (1), for PHBV aged in natural conditions (2) and for PHBV specimen aged in renewed and filtered seawater at 4°C (3), 25°C (4) and 40°C (5).

Fig. 5. Evolution of weight of loss as function of immersion time at different temperatures: natural ageing, 4°C, 25°C and 40°C.

Fig. 6. Evolution of weight loss as a function of roughness for the PHBV aged in immersion at different conditions.

Fig. 7. Evolution of the surface of PHBV samples aged in natural seawater.

Fig. 8. Evolution of tensile behavior of PHBV specimens aged in natural conditions and at 4, 25, and 40°C after 360 days: before (a) and after drying (b).

Fig. 9. Evolution of the strain at break of PHBV specimens aged in natural conditions and at 4, 25 and 40°C after 360 days: before (a) and after drying (b).

Fig. 10. Evolution of the stress at break of PHBV specimens aged in natural conditions and at 4, 25 and 40°C after 360 days: before (a) and after drying (b).

Fig.11. Evolution of the Young modulus of PHBV specimens aged in natural conditions and at 4, 25 and 40°C after 360 days: before (a) and after drying (b).

Fig. 12. Evolution of the molecular weight ($\bar{M}_w$) aged in natural conditions and at 4, 25, and 40°C in seawater after 360 days.

Fig. 13. Evolution of the stress at break (a) and strain at break (b) as a function of the molecular weight for PHBV specimen aged in seawater at different temperatures.

Fig. 14. Evolution of molecular weight ($\bar{M}_w$) versus water uptake (M_t) in seawater at different temperatures.

Fig. 15. Evolution of weight loss versus molecular weight in seawater at different temperatures.