

EFFECT OF ELECTRON BEAM IRRADIATION ON THE ENZYMATIC DEGRADATION OF COMPOSITES BASED ON BIODEGRADABLE POLYMERS AND COCONUT FIBER

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ABSTRACT

The development of polymeric materials that are susceptible to microbiological degradation and that have properties similar to the conventional polymers would reduce waste deposit. Degradable plastics suffer significant change on chemical structure when submitted to specific environmental condition. PCL and PLLA have been extensively investigated due to their bio-assimilation and because they are considered as eco-friendly. So the degradation of PCL and PLLA homopolymers, PCL:PLLA 20:80 (w:w) blend and coconut fiber-modified composites were studied by means of their degradation under lipase enzyme from *Pseudomonas cepacia*. Non-irradiated and EB-irradiated samples at 50 kGy and 100 kGy were exposed during 24, 72, 120 and 168 hours to the enzyme-buffer solution and the retained mass of dried samples was accompanied over time. The results were compared to the not submitted to the enzyme solution samples. Degradation rate of PCL was higher than PLLA in the presence of *Pseudomonas* lipase. PLLA presence reduced PCL's enzymatic degradation in the PCL:PLLA 20:80 w:w blend. After 120 h exposure, blend mass loss variation approached pure PLLA behavior. Composites degradation behavior through time was similar to the blend. Values of retained mass for composites were superior to the blends suggesting that coconut fiber did not significantly degrade in the period of test. Degradation rate of 50 kGy-irradiated PCL slightly reduced, and it was observed increase of degradation rate of samples irradiated with 100 kGy, probably attributed to its crystallinity decrease. Degradation rate of irradiated composite was similar to the blend, suggesting that fiber presence did not affect significantly this parameter. Samples tested during 168 h were affected by the water absorption by PLLA or coconut fibers through time testing. Studied samples degraded accentuatedly in the enzyme presence and were not negatively affected by the radiation processing.

1. INTRODUCTION

Developments of polymeric materials susceptible to microbiological degradation and which have similar performance to the conventional polymers have been studied in great extent. The intention is that those materials would reduce the residue deposit volume while undergoes to degradation on the sanitary waste deposit sites, or treated on the composting plants [1]. Liu et

al and Lenglet et al [2,3] have cited that PCL and PLLA biodegradability under natural conditions have been also investigated. In one side, PLLA is bio-absorbable, that is, byproducts of hydrolytic degradation can be completely by microorganisms as fungus and bacteria. On the other hand, PCL is readily biodegradable by microorganisms on the environment. So both PCL and PLLA can be considered as environmentally friendly polymers.

Kolybaba et al [4] cited that degradable polymers are those that suffer a significant change on the chemical structure under specific environmental conditions. Those changes result on both mechanical and physical properties loss, those changes are measurable by standard test methods. Biodegradable plastics undergo degradation by the action of microorganisms that naturally occurs as certain bacteria, fungus and algae. The plastic designed to be completely biodegradable fits among main category of polymeric materials. In this category polymeric matrix can be from natural sources and reinforcement fibers formed by vegetable fibers. Microorganisms are able to consume those materials completely, eventually releasing carbon dioxide and water as byproducts [4]. Composites of PCL, PLLA and coconut fiber studied here belong to this category.

According to Müller [1], there are different approaches concerning the kind of assay to be applied to evaluate the degradation of polymeric materials in the environment and which conclusions are obtained from that. At first they can be subdivided according to categories, field, simulation and laboratorial tests. Even though field test such as bury samples in soil, place in a lake or river, or perform general process of composting of the biodegradable polymeric material represent practical ideal conditions, there are several advantages related to this kind of testing. One of the concerns would be having the control of environmental conditions of degradation as temperature, pH or humidity. Other point that would be of concern is the necessity to monitor quantitatively the degradation process, in most of the cases it is possible to evaluate visually the sample modification, or perhaps to determine disintegration by measuring weight loss. Tests that are more reproducible are laboratorial ones, in which it is used defined media and inoculated with specific microorganisms to a particular polymer. In those cases enzymatic activity is optimized to a particular microorganism and frequently present degradation rate higher than what is observed in environmental conditions. That is considered an advantage to the study of the basic mechanisms of polymeric biodegradation. Even though results lead to limited conclusion concerning to the real degradation rate on the natural condition, those tests are widely used.

2. MATERIAL AND METHODS

2.1. Sample Preparation

PCL (pellets, $\bar{M}_w=2.14 \cdot 10^5 \text{ g}\cdot\text{mol}^{-1}$; $\bar{M}_w/\bar{M}_n = 1.423$), PLLA (pellets, $\bar{M}_w=2.64 \cdot 10^5 \text{ g}\cdot\text{mol}^{-1}$ $\bar{M}_w/\bar{M}_n = 1.518$ – Gel Permeation Chromatographic values) and dry coconut fiber (from *Embrapa – Empresa Brasileira de Pesquisa Agropecuária*, Ceará, Brazil) were used to prepare blends and composites. A Labo Plastomil model 50C 150 of Toyoseiki twin screw extruder was used for pellets preparation. Pellets of PLLA:PCL 80:20 (w:w) blend and composites containing 5 and 10% of untreated and chemically treated coconut fiber were prepared at AIST.

2.1.1. Hot pressed sheets

Sheets (150mm x 150mm x 0.5mm) of PCL, PLLA, PLLA:PCL 80:20 (w:w) blend and composites containing 5 and 10% untreated and chemically treated coconut fiber were prepared using Ikeda hot press equipment of JAEA. Mixed pellets of samples were preheated at 195°C for 3 min and then pressed under heating at the same temperature for another 3 min under pressure of 150 kgf cm⁻². The sample was then cooled in the cold press using water as a coolant for 3 min.

2.2. Irradiation

Hot pressed samples were irradiated in nylon/polyethylene bags sealed after removal of the air by a vacuum pump. The sheets were electron beam irradiated, EB, at JAEA using an accelerator with beam energy of 2 MeV and current of 2 mA, at a dose rate of 10 kGy pass⁻¹ with radiation doses up to 200 kGy.

2.3. Enzymatic Degradation

Buffered phosphate solution, pH 7, was prepared with lipase enzyme from *Pseudomonas cepacia* of Aldrich. Concentration of solutions was kept around 35 unities of enzymatic activity. It was used hot bath to maintain flasks at 37°C. Buffer-enzyme system was kept by 168h. Samples were tested by exposing them to buffer-enzyme system during 24, 72, 120 and 168 hours. After enzymatic exposure samples were washed, dried and their mass value obtained. Samples were tested in duplicate, and it was subtracted control without enzyme.

3. RESULTS AND DISCUSSION

By Fig. 1, it can be accompanied the retained mass for homopolymers, blend and composites during enzymatic degradation test. According to Liu et al [2], PCL enzymatic degradation of PCL has been investigated, mainly in presence of enzymes like lipase. It is well known that the morphology and its alteration play an important role in the hydrolytic degradation of aliphatic polyesters. When leading with enzymatic degradation, situation turns more complicated due to the specificity of enzymes. *Pseudomonas* lipase is able to break esters linkage in hydrophobic substrate, as it is the case for PCL. It is possible to observe in Fig. 1 that PCL degradation rate is superior to that of PLLA in the presence of *Pseudomonas* lipase, in agreement with what was observed by these authors that both crystalline and amorphous PCL can be degraded by lipase. Authors also observed that PCL has not absorbed water, while PLLA absorbed approximately 2% of water in 72h. Authors also observed that degradation rate of this polymer is higher in the presence of proteinase K than in lipase (8% against 1%). However, in this study it was observed that PCL degraded 30% and PLLA 16% in the same time.

According to Tsuji and Ishizaka [5], it was not observed changes on the molar mass distribution neither weight loss of pure PLLA films studied by them, indicating that the effect of enzymatic hydrolysis by *Pseudomonas* lipase on PLLA chains in the polymeric matrix was not significant. This confirms that enzymatic degradation preferably occurs on the sample surface.

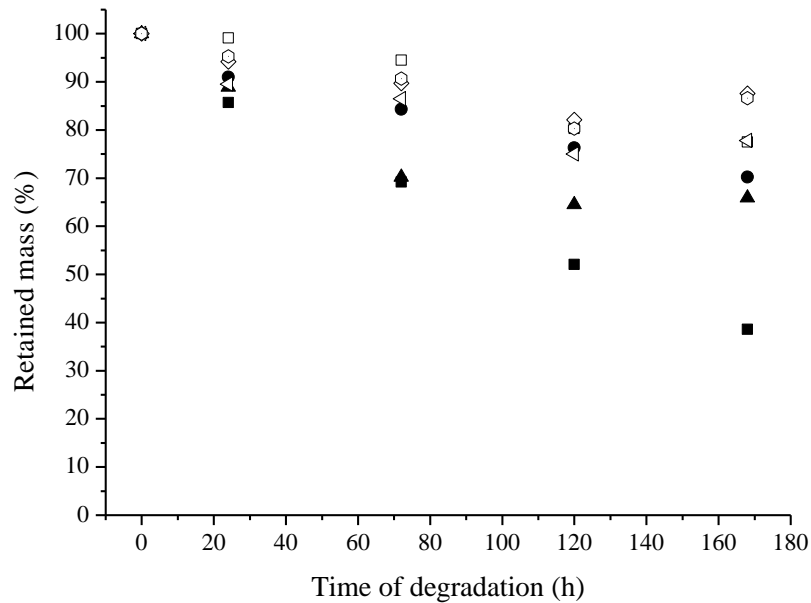


Figure 1. Retained mass as a function of degradation time from non irradiated samples of: (■) PCL; (●) PLLA; (▲) PCL:PLLA 20:80; (□) composite with 5% of non treated fiber; (◇) composite with 10% of non treated fiber; (◁) composite with 5% of acetylated fiber; and (○) composite with 10% of acetylated fiber.

Calil et al and Sivalingam et al [6,7] have cited that the presence of one polymer affects the degradability of the other. Lenglet et al [3] observed that the addition of PLLA to PCL reduced drastically PCL degradation of blends in presence of lipase. In this study, it was possible to observe that PLLA presence reduced enzymatic degradation of PCL of the PCL:PLLA 20:80 (w:w), and after 120 hours, retained mass variation approached the behavior of pure PLLA. Tsuji e Ishizaka [5] studied enzymatic degradation of PCL:PLLA blends using *Rhizopus arrhizus* lipase. The authors observed that enzymes from fungus cause selective hydrolysis and PCL removal from PCL:PLLA blends without causing significant biodegradation of PLLA in soil. The authors cited that enzymatic hydrolysis of PCL:PLLA in presence of *Pseudomonas* lipase and proteinase K occurred in the interface between two polymeric phases, both internal and superficial areas of samples. The authors observed that PCL crystallinity in the blends films did not change with variation of composition during degradation, suggesting that this property did not affect enzymatic hydrolysis of PCL in the blend. Enzymatic hydrolysis rates of blends were lower compared to pure PCL, suggesting that PLLA interfered on PCL hydrolysis catalyzed by lipase. One reason postulated by the authors for the degradation deceleration of PCL in blends was because the disturbance caused by superficial adsorption of enzyme molecules on the films or due to the slow hydrolytic scission of PCL main chain by enzymes molecules in presence of PLLA molecules in blends.

In this study, composites degradation behavior was similar to the blend through the same period of time. Retained mass values were superior to the blend for the same period of measurements, This suggested that coconut fiber did not suffered significant enzymatic degradation in the condition of testing. Acetylation treatment of fibers seemed not affect

significantly composites enzymatic degradation. Fig. 2 presents attack points of lipase on polyesters, as proposed by Sivalingam et al [7].

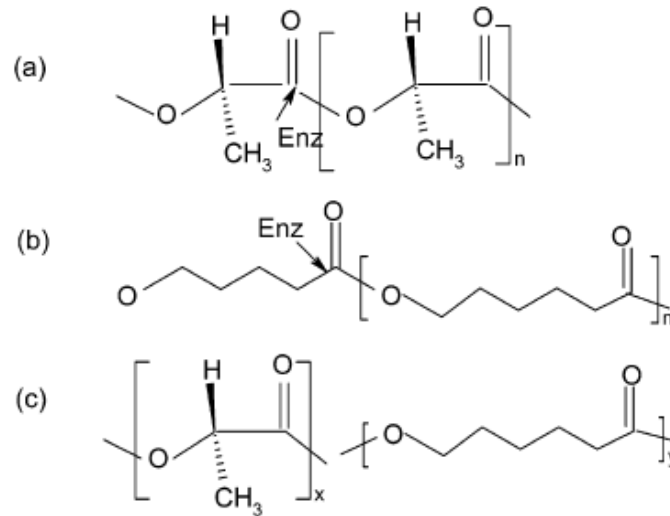


Figure 2. Attack point on polyesters with lipase: a) PLA; b) PCL; c) PCL:PLA 14:86 (Sivalingam et al, 2004)

It is possible to observe in Fig. 3 radiation dose effect on enzymatic degradation of PCL samples irradiated with EB.

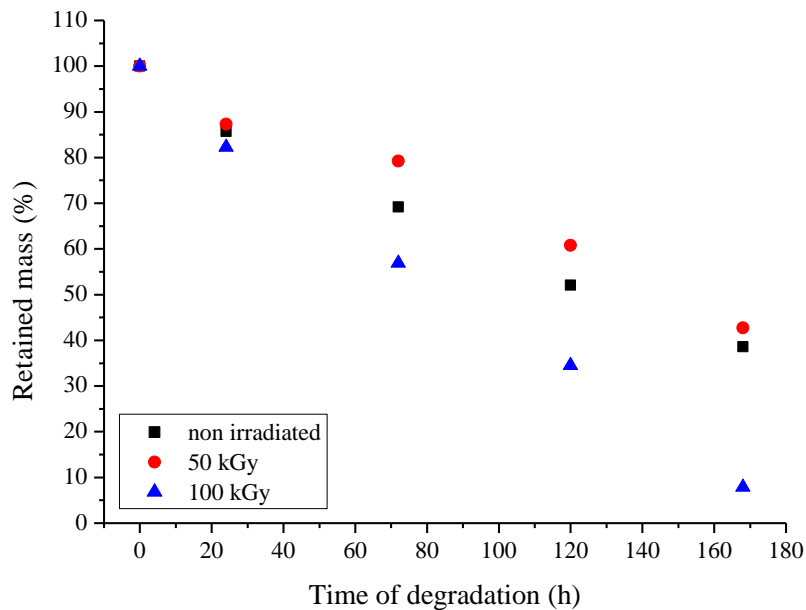


Figure 2. Retained mass as a function of degradation time from non irradiated PCL and EB irradiated PCL samples.

According to Cottam et al [8], degradability rate of PCL irradiated with 25 kGy decreased, that was attributed to the radiation process. The authors cited that lipase catalyzes the hydrolysis of carbonyl group and one carbon atom in case of fat. It is the same bond that is

broken during PCL hydrolysis. The authors considered that PCL degradation rate was affected by radiation induced cross-linking. In this study, PCL irradiated at 50 kGy presented slight decrease on degradation rate, in agreement to authors' observation. On the other hand, PCL samples irradiated with 100 kGy suffered certain increase of the degradation rate. This fact probably is related to crystallinity reduction of about 6% previously observed by DSC [9,10].

The effect of radiation dose on the enzymatic degradation of EB irradiated PLLA samples can be observed in Fig. 4.

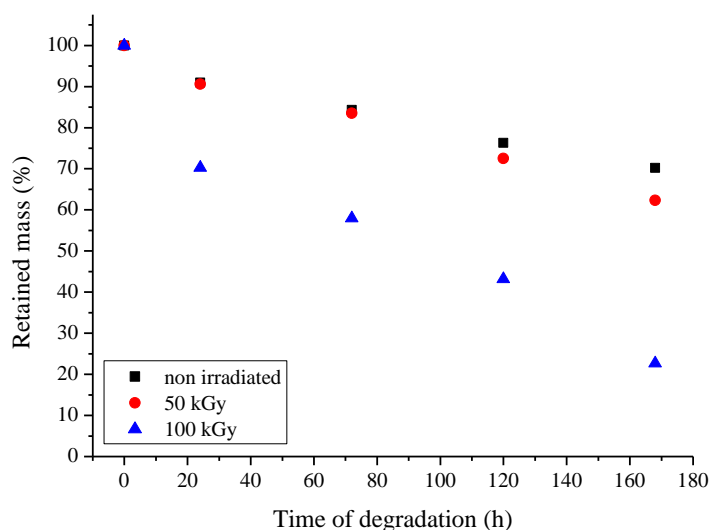


Figure 3. Retained mass as a function of degradation time from non irradiated and EB irradiated PLLA samples.

According to Maharana et al [11], enzymatic degradation occurs only on the surface of a solid substrate by erosion and mass loss, because enzymes are not able to penetrate polymeric solid substrate. Enzymes degrade selectively amorphous regions or less ordered ones that allows them to diffuse through substrate and, subsequently, crystalline regions are eventually degraded. In this process, molecular weight and molecular weight distribution of non degraded solid substrate do not change during degradation because only the polymer on the surface of substrate is degraded, and degradation products with low molecular weight are removed from substrate by solubilization in surrounding aqueous medium. There are two kinds of degradation based on cleavage point. Cleavage can occur in random points along the polymer chain (endo-type degradation) or at the end of the polymer chain (exo-type degradation). Lipases degradation process is based on endo-type and, so, do not depend on molecular weight and molecular weight distribution. In Fig. 5 it is presented the hydrolysis reaction cited by Maharana et al [11].

Retained mass with period of time of enzymatic degradation of PCL:PLLA 20:80 (w:w) blend, non irradiated and irradiated with 50kGy and 100kGy radiation doses is shown in Fig. 6. Results in this study are lower than reported previously in the literature, probably because samples studied here are physical mixtures of the polymers. Lenglet et al [3] studied enzymatic degradation of PCL:PLLA copolymers with \bar{M}_n around 29000 to 44000, in the presence of *Pseudomonas* lipase. Authors observed that with higher amount of PCL, degradation occurred faster, achieving around 99% for PCL:PLA 75:25 after 72 hours of

testing. Authors suggested that PCL homopolymer degrades in the presence of *Pseudomonas* lipase, meanwhile, PLA do not in the same conditions.

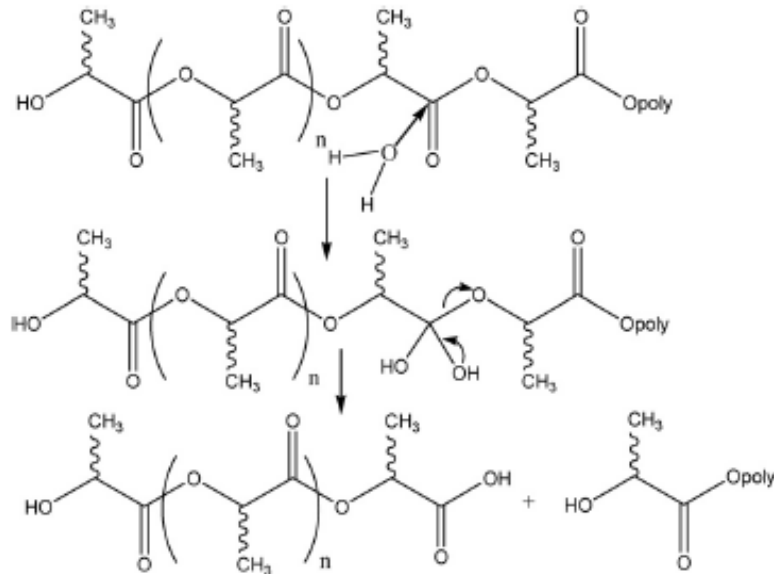


Figure 4. PLA hydrolysis [11].

In this study, Fig. 6, PCL:PLLA blend irradiated with 50 kGy presents slight degradation rate reduction compared to non irradiated blend, in agreement with that was observed in homopolymers. Irradiated sample with 100 kGy shows slight increase of the degradation rate after 120 hours, and after that degrades just a few.

Retained mass with degradation time of the composites with 5% of non treated fiber, non irradiated and irradiated with 50 kGy and 100 kGy is shown in Fig. 7.

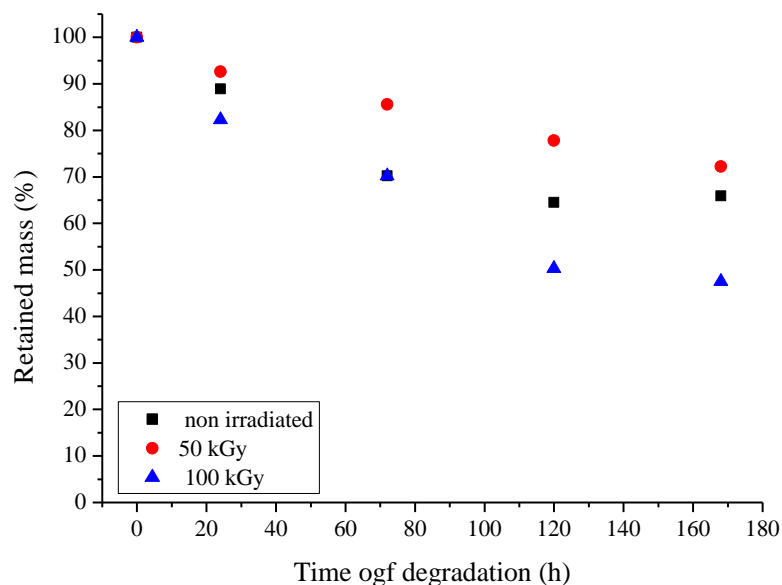


Figure 5. Retained mass as a function of degradation time from non irradiated and EB irradiated PCL:PLLA 20:80 (w:w) samples.

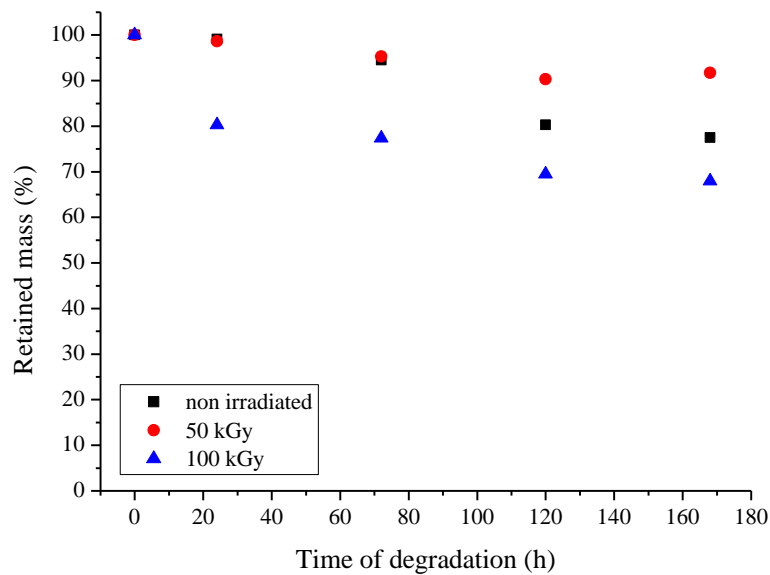


Figure 6. Retained mass as a function of degradation time from non irradiated and EB irradiated of composites with 5% of non treated fiber, non irradiated and EB irradiated.

Coconut non treated fiber addition caused slight reduction of degradation when comparing the blend and the composite. Probably it happened because fibers take longer to degrade. Even though a different method than used here was used by Salazar and Leão [12], the authors observed that coconut fiber *in natura* degraded 10% in 912 hours (38 days) by testing immediate degradation measuring carbon dioxide released in open system, in which an organic substance is submitted to metabolism by a mixed culture of microorganisms from the environment. This carbon source from the substance can be completely consumed by microorganism metabolism converted to CO₂ and H₂O. It is possible to predict theoretically total CO₂ production to total complete biodegradation, it is necessary to know the initial carbon content.

Degradation rate behavior shown in Fig. 7 is similar to irradiated blends, suggesting that fiber presence did not affect this parameter. Retained mass values of the samples tested in 168 hours were probably affected by water absorption by PLLA and coconut fibers.

It can be observed in Fig. 8 that as non treated coconut fiber content increases in the composite, degradation rate slightly reduces and degradation decreases with increasing radiation dose.

Retained mass with time of degradation of composites with 5% of acetylated fibers, non irradiated and irradiated with 50kGy and 100kGy radiation doses is shown in Fig. 9. Acetylation process of coconut fibers did not affect significantly degradation rate of samples. Irradiated samples enzymatic degradation slightly reduced compared to non irradiated composite with 5% of acetylated fiber. The increasing of acetylated fiber content up to 10 % neither affected degradation rate nor degradation of irradiated composites with 50 kGy and 100 kGy.

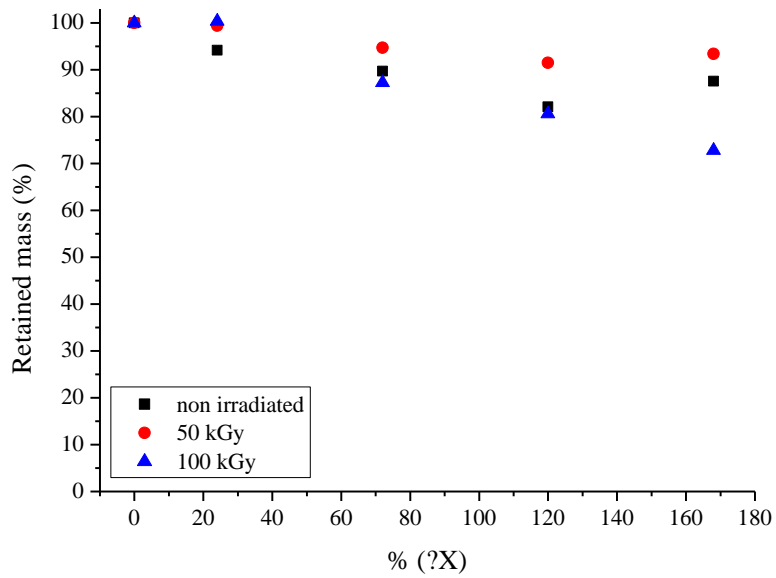


Figure 7. Retained mass with degradation time of non irradiated and EB irradiated of composites with 10% of non treated fiber, non irradiated and EB irradiated.

After 168 hours it was observed slight increase of retained mass, probably because water absorption by PLLA or coconut fibers, Fig. 10.

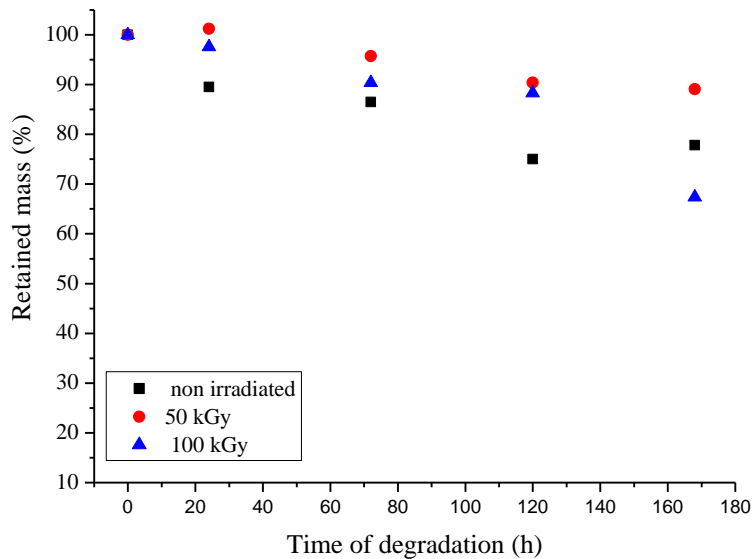


Figure 8. Retained mass as a function of degradation time from non irradiated and EB irradiated of composites with 5% of acetylated fiber, non irradiated and EB irradiated.

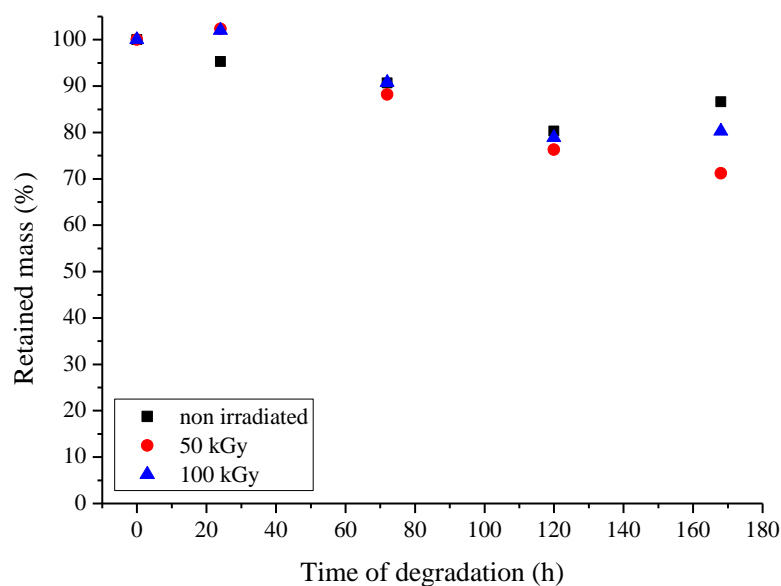


Figure 9. Retained mass degradation time of non irradiated and EB irradiated of composites with 10% of acetylated fiber, non irradiated and EB irradiated.

3. CONCLUSIONS

Studied materials degraded in presence of enzymes and they were not negatively affected by radiation processing. Even though fiber addition reduced slightly the degradation process, composites carried degrading through time. Products produced using the material studied here can be irradiated up to 100 kGy radiation doses, without any prejudice to their biodegradability.

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