2021 年臺灣國際科學展覽會
優勝作品專輯

作品編號　100043

參展科別　工程學

作品名稱　Biodegradation of Post-Cured Photopolymeric Resin of Stereolithography 3D Printers Using Galleria mellonella Larva.

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關鍵詞　Photopolymer、Biodegradation、Galleria mellonella

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ABSTRACT

The present research has as main objective to degrade the post-cured photopolymer of the stereolithography 3D printer resin using Galleria mellonella larvae. It is necessary to consider that the use of materials from 3D printers tends to increase considerably and in approximately seven years about 10% of everything that will be produced in the world will come from this type of printing. Considering also that the increase in population growth and technological development are directly linked to the increase of solid waste on the planet, in particular to polymeric materials, there is a need to degrade and give an adequate end to waste, avoiding a notorious accumulation along the time. For this purpose, Galleria mellonella larvae will be used because of it's comprovated capacity to degrade polyethylene, to find out if it is capable of biodegrading the post-cured resin of the printer. To carry out the research, compositional tests were done in partnership with the SENAI Institute for Innovation in Polymer Engineering, located in São Leopoldo, Rio Grande do Sul, and the creation of the larvae and degradation of the photopolymer will be carried out in partnership with the University Federal University of Health Sciences of Porto Alegre (UFCSPA). The data analysis will be based on the crystallinity determination tests by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and attenuated total reflectance spectroscopy (ATR) that will also be applied in the larvae feces after contact with the polymer to assess for degradation. As a result of the compositional tests, the ATR showed predominantly characteristic absorptions of acrylic resin; in the TGA test, the loss of mass described in the test is related to the loss of mass of organic material, mainly polymer. Finally, in the DSC test a thermal event was observed in the heating of the sample, with peaks at 125 ° C (Tpm), characteristic of fusion, and a thermal event in the cooling of the sample, in 112 ° C (Tpc), characteristic of crystallization. Based on the analysis of the results obtained, it is possible to infer that most of the composition of the photopolymer is acrylic resin, widely used in stereolithography 3D printers. The research has the future objective of isolating the substance into the larvae responsible for degradation so that it can be degraded on industrial scales. The research started in March 2020 and is still under development due to the COVID-19 pandemic, which compromised the planned tests.

OUR THANKS

In this trajectory as students and researchers, we had the opportunity to collect many apprenticeships that were reflected in personal and professional maturation, and many of them are materialized through this project. We therefore recognise that we have a lot to thank, because this work is the result of joint efforts.

First of all, we would like to thank our families, who encouraged us to study and timed a technical course of such quality. The days studying until dawn, the difficulties and moments when we need comfort with certainty were worthwhile, and without the support of you none of this would be possible.

We also thank our teachers, who from the first contact with research instigated us to curiosity and critical gaze, without such learning we would never seek to change and evaluate the environment in which we live with such clarity and determination. Especially our mentor Schana and project contributors Gislene, Karin and Danielle, who embraced this project with us and made it possible.

We thank our friendships, which were the friendly shoulder in moments of frustration and tiredness, moments when we felt like giving up and after long conversations and good laughter, everything seemed to improve. Surely this project is also due to this.

Finally, we thank the organization of all the fairs that we participate for expecting an exchange of knowledge and improvement of our ideas to the point of taking us further in the name of science. Farther than we thought possible and farther than we thought we could get.

This project is the result of much dedication, and reflection of two grateful hearts ready to face the next challenges with an even more curious and hopeful look.

1. INTRODUCTION

It is known that the use of plastics is of paramount importance in the most varied areas of application, and to this end, the creation of synthetic polymers makes projects feasible and makes the use of these materials more accessible. As an example, we have the 3D printers that, according to the report The Future of Work (2018), of the World Economic Forum, will be used in 49% of Brazilian companies in different areas by 2022. To this end, different machines with various materials are used, but the 3D stereolithography printer is highlighted because, according to NASCIMENTO (2020), this method presents rapid prototyping and with high precision, being a preferred economic option for a wide variety of industries, including automotive, medical, aerospace, entertainment and is also used for the development of consumer products. Moreover, according to research conducted by Almeida (2018), it is estimated that in approximately seven years, about 10% of all that will be produced in the world will come from this type of printing.

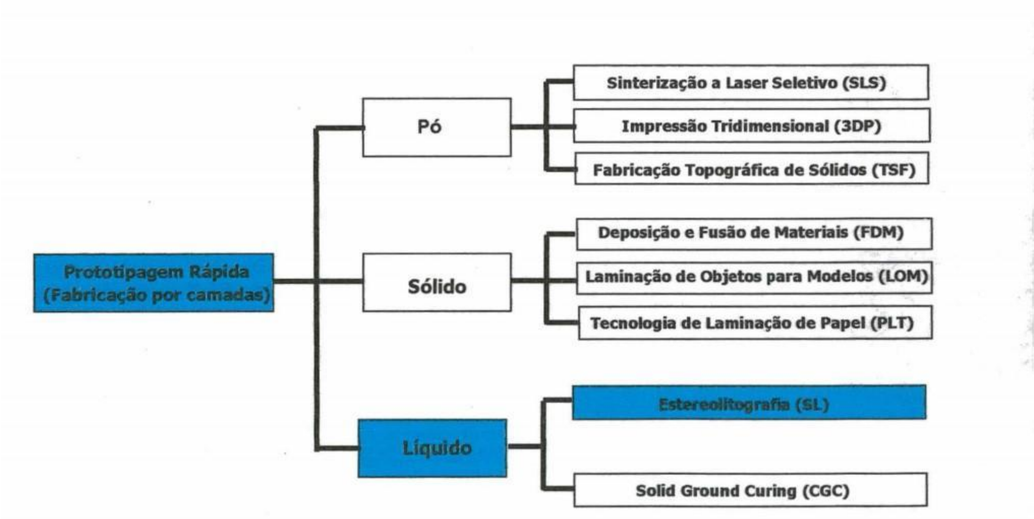
Despite the protagonism of the material, the increase in population growth and technological development are directly linked to the increase of solid waste on the planet, in particular to polymeric materials (Miranda, 2016), thus generating an undue disposition and notorious accumulation of these over time. The disposal of solid waste is a problem that keeps pace with industrial developments and is increasingly worsening.

As an alternative to solving this problem, a 2017 survey by BOMBELLI, HOWE and BERTOCCHINI evaluated that the Galleria mellonella larva, which is a parasite insect of hives, was able to biodegrade polyethylene, a thermoplastic. Therefore, from this, it is possible to question whether the Galleria mellonella larva acts in the process of biodegradation of the post-curated photopolymeric resin of 3D stereolithography printers, because, despite this material has excellent handling properties and industrial use, it is difficult to degrade. Therefore, the project aims to analyze whether the Galleria mellonella larva is capable of degrading the post-curated photopolymeric resin of 3D stereolithography printers. Due to the facts mentioned, it is suggested that the insect will be able to biodegrade the photopolymer and that such biodegradation will be viable economically, due to the fact that the Galleria mellonella larva is of easy creation and great abundance, being used as bait for fishing, besides being a parasite of hives (VIRTUAL, 2017).

2. BIBLIOGRAPHIC REFERENCE

2.1 3D stereolithography printers

According to the requirements related to prototypes and their purposes, various types of rapid prototyping processes have been developed. According to FERNANDES (2001), some processes are more employed than others due to factors such as prototype performance, construction speed and process cost. One of the best ways to classify the numerous rapid prototyping systems is by the initial form of the material used, powder-based, solid or liquid, as explained in the image below:



(FERNANDES, 2001, p. 5 - Figure 1)

Considering the characteristics presented, the focus is on the 3D Sterolithography Printer (SLA), which, according to 3D SYSTEMS (2020), uses a cube of liquid photopolymer resin cured by an ultraviolet laser (UV) to solidify the layer-to-layer pattern, creating a solid 3D model from data 3D developed graffiti SLA printers are capable of printing highly detailed parts ranging from a few millimeters thick to 1.5 meters (5 feet), with great precision and resolution. Also, according to RODRIGUES (2018), 3D Systems created, in 1992, the world's first SLA device, which enabled the manufacture of complex parts, layer by layer, in a fraction of the time it would normally take.

According to FERREIRA et al. (2000) prototypes are developed independently of the geometric complexity of the part by photopolymerizing epoxy-based resins by a beam of ultraviolet laser rays, the movement of which is controlled by a computational program.

2.2 Photopolymers

According to Portal San Francisco (2020), a photopolymer is a polymer that heals, or becomes solid, when exposed to light. This light, or actinic radiation, can be emitted by a laser or a lamp. In general, a photopolymer consists of a complex mixture of compounds rather than a single element, and is the raw material of 3D stereolithography printers. According to FERNANDES (2001), photopolymerization is the process of radiation polymerization, where bonds occur between small molecules (monomers) forming a large molecule (polymer) composed of several monomeric units.

According to FERNANDES (2001), vinyl monomers, which make up photopolymers, are defined as monomers containing a double carbon-carbon bond. The vinyl group may still be connected to another group of molecules, which may also contain other vinyl groups, in which case the monomer is called multifunctional. The polymerization of multifunctional monomers results in a polymer with cross bonds and due to the existence of cross bonds, these once formed materials retain their three-dimensional shape and cannot be shaped in later processes, besides not having solvent solubility. The degree of cross-links provides specific properties such as high hardness, dimensional stability and fragile behavior at room temperature.

According to FERNANDES (2001), acrylates are the most used class of resins in the printing industry and UV-curable coatings, however, according to Portal San Francisco (2020), polymer bases for photopolymers include acrylics, polyvinyl alcohol, polyvinyl cinamate, polyisimers. Nevertheless, photopolymers, also referred to in trade as photopolymer resins or UV resins that are used for stereolithography, are based on epoxy and acrylic chemistry.

2.3 Galleria mellonella

The Galleria mellonella, a species of lepidopteran insect (Greek lepis, scales, and pteron, wings), is also known as the large wax moth or the honeycomb moth. It belongs to the family Pyralidae and is the only member of the genus Galleria. It is assumed that Europe is its native origin and that the species has been introduced to other continents such as North America and Australia (CAMARGO, 2015), but currently it can be found worldwide.

The insect has a life cycle of seven weeks and it takes five weeks for the egg to develop to the last stage as a larva. After that, it takes two weeks until it evolves into an adult moth. Females and males are distinguished according to size (10-15 mm for males and 20 mm for females) and color (females tend to be darker than males). For the breeding of the larvae in the laboratory, it is necessary to place adult moths in containers with lid and paper for laying the eggs. Females lay their eggs on the edges of the papers after two days. Then the eggs are removed and placed in plastic boxes with perforated lids, thus allowing air to enter. The eggs hatch in three days and the larvae are fed both with pollen and beeswax as well as with an artificial diet, which can be composed of liquid honey, glycerin and a mixture of flours (wheat flour, corn, beer yeast and milk powder). When they stop feeding, they reach the sixth stage, which is the formation of the pupa in which they remain for two weeks. After this, they become adult moths, completing their life cycle (RAMARAO; NIELSEN-LEROUX; LERECLUS, 2012).

2.4 Types of degradation

According to REIS et al. (2006), one of the factors that differentiates a biodegradable polymer from a non-biodegradable polymer is the period of time that the degradation process takes to occur. The degradation of a polymer is defined as a process of division of the polymer chain during which it is broken into smaller segments (oligomers), and later into monomers again.

According to GIORDANO and CAMPOS (2000), the concept of degradation in polymers is associated with any chemical reaction that causes undesirable changes, which leads to a loss of material properties. Polymeric degradation can be both structural and superficial. The first refers only to the surface, thus modifying the visual aspect and the second generates changes in mechanical, electrical and thermal properties, compromising its structural performance. According to GIORDANO and CAMPOS (2000), degradation reactions can be divided into: thermal, chemical, mechanical, biological, thermooxidative, photooxidative, thermomechanical, mechanochemical, among others. Such degradation processes may be reduced when stabilising elements are used.x

3. METHODOLOGY

The research can be classified in applied, because, according to Prodanov and Freitas (2018, p. 51) aims to generate knowledge for practical application directed to the solution of specific situations. Also for Prodanov and Freitas (2018, p. 52), from the point of view of the objectives it is possible to consider the research as exploratory, because it is in the preliminary phase and aims to provide more information on the subject that will be investigated, enabling its definition and its delineation, that is, to facilitate the delimitation of the topic of the research; to guide the setting of objectives and formulation of hypotheses or to discover a new type of approach to the subject. From the point of view of the procedure used, the project is considered experimental, as it seeks to remake the conditions of a fact to be studied, to observe it under control. For this purpose, an appropriate location, apparatus and precision instruments are used to demonstrate the way or causes by which something is produced, thus providing the study of its causes and effects. Finally, from the point of view of the problem approach, the research is considered quantitative, since it considers that everything can be quantifiable, translates in numbers opinions and information to classify and analyze them, requiring the use of resources and statistical techniques (Prodanov and Freitas, 2018, p. 69).

For the application of the research, a sample of photopolymeric resin post-curated with about 10g provided by the company Elton Matrizes Ltda., located in New Hamburg/RS, was submitted to thermogravimetry analysis. (TGA), determination of crystallinity by differential scanning calorimetry (DSC) and infrared testing through Mitigated total reflectance spectroscopy held at the SENAI Institute of Innovation in Polymer Engineering, São Leopoldo/RS. The initial tests aimed to determine the composition of the sample, so that after contact with the larva it is possible to investigate if there was degradation. Similarly, all components identified in the resin composition will be placed in contact with Galleria mellonella larvae separately to identify whether there is a dietary preference for a given compound.

3.1 Thermogravimetric analysis

Thermogravimetry (TGA), according to Thermal Analyses (2020), is a technique in which the variation of the mass of the sample (loss or gain) is determined according to temperature and/or time, while the sample is subjected to controlled temperature programming. The trial was conducted at the SENAI Institute of Innovation in Polymer Engineering and the equipment has exclusive software that allows the direct acquisition of the data as well as the monitoring of the test. According to data from the literature presented by ESTEVES et al. (2017), thermogravimetric curves were obtained using three different heating rates (3, 5 and 10 K min-1) in the range of 300 K to 975 K, under compressed air atmosphere and with flow maintained at 20 mL/min. Open platinum chains were used. The TGA trial was conducted according to UL 746 A standards, item 45 ASTM D 3850, available on the website of the Senai Institute of Innovation in Polymer Engineering (2020). Also, according to Lodi, Bueno and Zornberg (2009), the temperature range used ranged from room temperature (near 23 ° C) to temperature of 700 ° C in a saturated atmosphere of nitrogen. This final temperature is sufficient to oxidize the substances present in the samples.

3.2 Differential Scanning Calorimetry (DSC)

According to Canevarolo (2006), the equipment consists essentially of a measuring cell in which the sample and a reference material are placed, means of controlling the temperature of the cell and other instruments of control and measurement. Normally, a constant rate of temperature variation is used, continuously measuring the property of interest. According to the facts, a differential scanning calorimeter was used operating at a heating or cooling rate of 10 ° C/min, from room temperature up to 300 ° C. Afterwards, the sample of post-cured photopolymer was subjected to a standard thermal cycle. This consists of a heating from the initial Ti temperature (usually ambient) at constant rate up to Tf = Tm + 20 ° C, performing isothermal treatment for 5 minutes and cooling at constant rate up to the initial temperature and second heating at constant rate (usually equal to that used in the first heating) up to Tf. Using the equipment software, it was possible to determine the melting temperature and enthalpy of all the peaks, drawing the thermograms, noting the value of the transition temperatures and their melting enthalpy.

3.3 Total attenuated reflectance spectroscopy (FTIR)

According to the specification of the equipment, available by the USP (2016), the PSO is a technique used to obtain FTIR spectra from samples of difficult handling. The test is based on the reflection suffered by a beam of radiation when it passes from a denser medium to a less dense one. The technique uses a crystal of high refractive index, which allows the total internal reflection of the infrared beam when it passes from the crystal to the sample. Before reflection the beam penetrates slightly into the less dense material forming an evanescent wave. The sample can then absorb some of the evanescent radiation causing the attenuation of the beam intensity in the absorption wavelengths. To this end, infrared spectrometry has been used for qualitative purposes, to obtain information about the functional groups present and for presumptive identification by comparison with spectrum banks, such as the Sadtler Database or to follow organic reactions by monitoring certain absorption bands. In order to carry out the experiment, according to the FITR Equipment Technical Data Sheet, it was used the ModulI and in the spectrometer, in addition to the support with the sample, and 800 μL of commercial formaldehyde, free of methanol, were added in the ATR support closing the system and taking the spectrum in absorbance mode. This test, as well as the previous ones, was conducted at the SENAI Institute of Innovation in Polymer Engineering under the supervision of the project contributors Gislene Zehetmeyer and Karin Janete Stein Brito.

3.4 Next Steps

Due to the COVID-19 pandemic, the degradation of photopolymer by the larvae was compromised because the Federal University of Health Sciences of Porto Alegre (UFCSPA) is closed. Currently the data concerning the composition of the photopolymer have allowed the analysis of hypotheses about the way the degradation will occur based on previously published research, evaluating the behavior of the polymer and organism of the larvae. After the analysis and interpretation of the graphs for the determination of the composition of the post-curated resin, a residual sample of photopolymer collected in the company Maquette Labs will be pressed in hydraulic press in the company Elton Matrizes Ltda. for a film with a thickness of 3mm and with sides of 5cm each. The film obtained will be taken to the Federal University of Health Sciences of Porto Alegre (UFCSPA), where it will be placed in contact with the larvae Galleria mellonella.

G. mellonella larvae will be grown in the UFCSPA molecular microbiology laboratory and throughout the life cycle the larvae will be kept at 28 ° C. Before the experiments, the larvae will be fed with artificial ration, which is composed of wheat flour, coarse wheat flour, wheat germ, powdered milk, liquid honey, glycerol and chewing sugar. When they reach the ideal weight for the experiments, groups of larvae will be fed with ration and the other groups with the polymer samples to be evaluated, for comparison.

An analysis of the consumption of polymer by larvae will be carried out. When the Galleria mellonella reaches a weight of approximately 150 mg, being weighed in analytical scales daily, each larva will be individually placed in a glass tube with a thread cap in contact with the previously heavy polymer film, and by difference in weight the individual consumption will be calculated after approximately 24 hours. The photopolymer, as well as each component of the sample, will be placed in contact with the larvae in triplicate. Subsequently, the larvae will be cleaned using alcohol 70 degrees, collecting the faeces in clean and dry glass tubes with threaded lid.

The feces of the larva in solution will be forwarded to the SENAI Institute of Innovation in Polymer Engineering, to carry out the TGA tests and, subsequently, will be forwarded to the Liberato Foundation to carry out the infrared test in A module, following the same procedure described above. The results obtained will be analyzed to determine, through the composition of the faeces, if there has been degradation of the polymers analyzed.

4. RESULTS AND DISCUSSION

The sample analysed was gray coloured and drilled in 3 regions for DSC, TGA and FTIR tests. The regions identified in the tables by R1, R2 E R3 are specified below:

Region 1

Photo taken by the project contributor Gislene Zehetmeyer - 06/01/2020

Region 2

Photo taken by the project contributor Gislene Zehetmeyer - 06/01/2020

Region 3

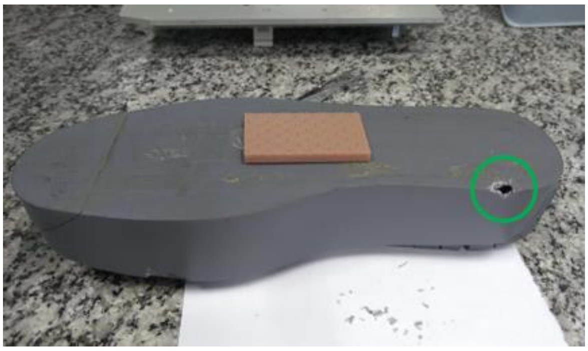


Photo taken by the project contributor Gislene Zehetmeyer - 06/01/2020

4.1 FTIR

The spectrum in the infrared of the sample predominantly exhibits absorptions characteristic of acrylic resin (see ANNEX 1) and other lower intensity absorptions present in the spectrum indicate the presence of some other material in which it is not possible to identify due to the low intensity of the absorption bands.

4.2 DSC

A thermal event was observed in the heating of the sample, with peaks at 125 ° C (Tpm) characteristic of fusion. A thermal event was observed in the cooling of the sample, at 112 ° C (Tpc), characteristic of crystallization, being:

1) Tpc: Crystallization temperature obtained at peak.
2) Tpm: Melting temperature obtained at peak.

DSC Results

Gray Sample	Tm (ºC)	ΔHm (J/g)	Tc (ºC)	ΔHc (J/g)
R1	126	4,4	112	3,1
R2	125	4,3	112	3,1
R3	125	4,5	111	3,2

4.3 TGA

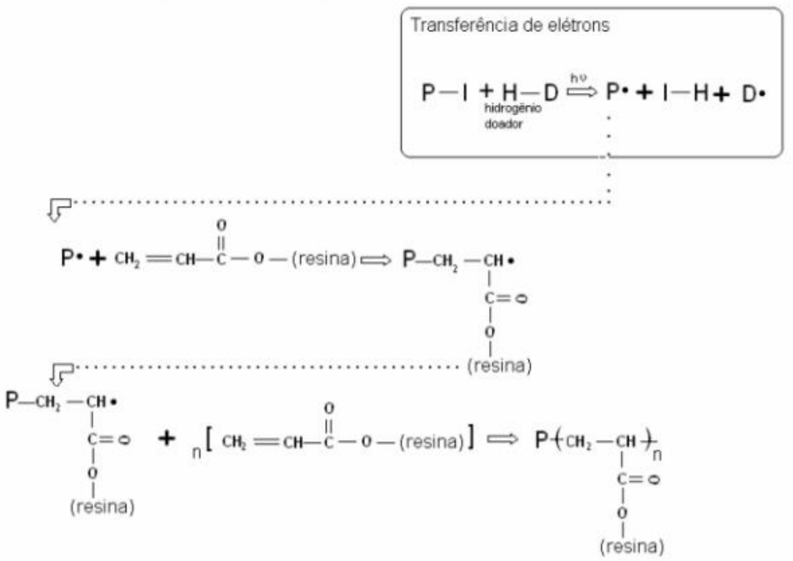
The loss of mass described in the test is related to the loss of mass of organic material, mainly polymer. Residues already refer to inorganic materials, such as metal oxides and inorganic loads assigned to the sample.

TGA Results

Grey Sample	Mass loss from 28°C to 900°C (%)	Waste at 900°C (%)
R1	99,5	0,5
R2	98,2	0,8
R3	99,6	0,4

4.4 Analyses

According to the tests carried out at the SENAI Institute of Innovation in Polymer Engineering, São Leopoldo/RS, the polymer is in its vast majority acrylic resin (see ANNEX 1). Acrylic resins are considered noble resins, of excellent chemical resistance, thanks to carbon-carbon bonds, much more resistant than ester bonds present in alkyid resins and polyesters (CORREA, 2003). Acrylates are chemicals known to easily polymerize when exposed to appropriate radiation in the presence of free photoinitiator radicals, characterized by the presence of an atom containing only one electron in a given orbital (apud. KLAUSS, 2006). Acrylic resins used in 3D stereolithography printers undergo a free radical polymerization process, as exemplified by the following scheme:



Polymerization by free radicals of an acrylate resin (WEISS, 1997)

The mechanism of photopolymerization by UV rays can be explained by JACOBS (1996) according to the scheme below, in which the photoinitiator molecules, P, present in the material are mixed with the monomers, M and absorb photons at a given frequency, coming from the incidence of the laser of the stereolithography printer, A fraction of P * is converted into a reactive (free radical) initiating molecule, P •, after passing through several steps of transformations. These molecules then react with a monomer molecule to form a polymerization initiator, P - M •, thus initiating the process of polymeric chain formation. The additional monomers continue to react during the propagation of the chain, PMMMMM •, until the terminal process of polymerization. As reactions occur, the resin becomes solid, increasing its mechanical strength, density and reaching the desired properties.

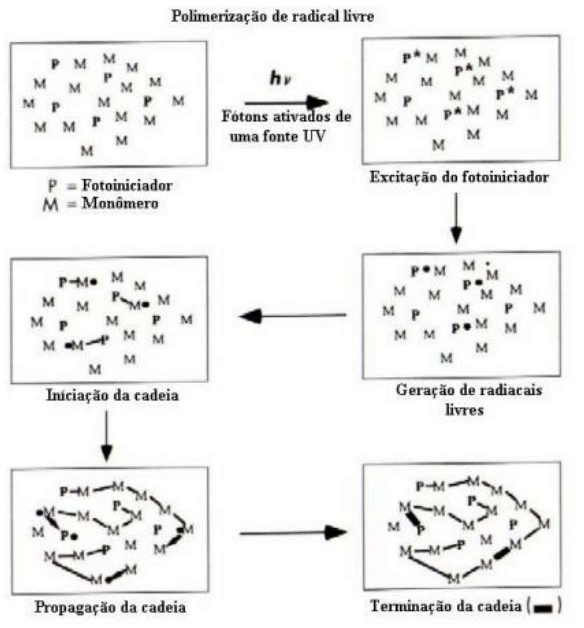
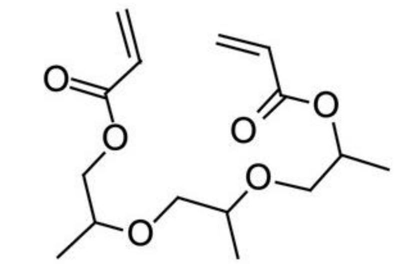
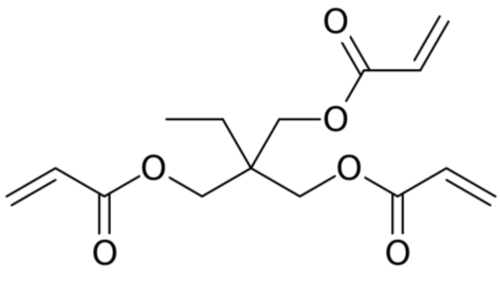


Illustration of the reaction sequence during photopolymerization (FERNANDES, 2003).

According to the added acrylic monomer, the polymer will acquire a certain structure. The most commonly used monomers are:





5. CONCLUSION

Based on the analysis of the structure of the post-cured photopolymeric resin sample, identified as acrylic resin, it is possible to raise hypotheses about how the larva will behave in relation to the polymer based on pre-conceived research. Through the work of BOMBELLI, HOWE and BERTOCCHINI developed in 2017, it is possible to observe that the *Galleria mellonella* larva was able to biodegrade low density polyethylene. Polyethylene is an inert material that is widely resistant to biodegradation and is rapidly consumed by Galleria mellonella larvae. This degradation process did not occur due to chewing of larvae, but through microorganisms or enzymes contained in your body. Spectroscopy in the infrared region revealed that low density PE contains branched chains, as well as acrylic resin. Therefore, considering that the branches were not a stumbling block for the larva to degrade polyethylene, Galleria mellonella would also be able to degrade the branches contained in the acrylic resin.

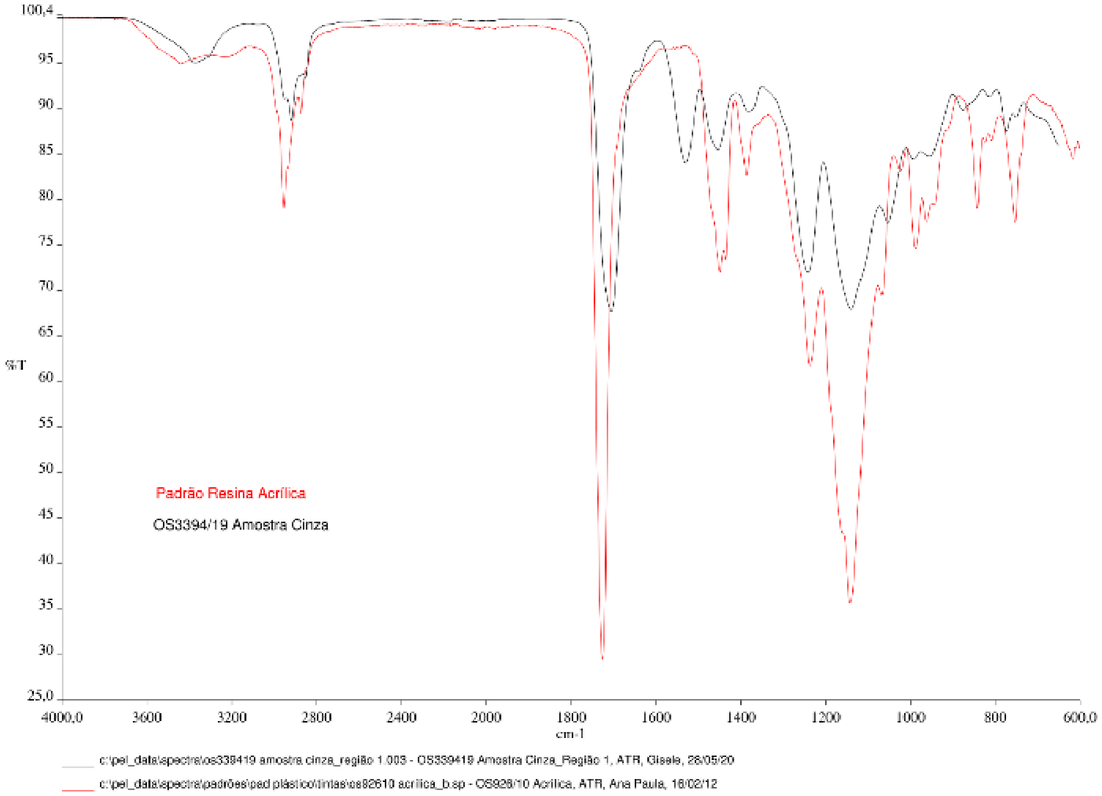
Another hypothesis raised is that considering the possible presence of hydroxyl groups in the resin structure (see ANNEX 2), degradation will be accelerated as it has been reported that better organic degradation efficiencies can be achieved by the presence of hydroxyl radicals (Neto, 2018), besides that according to RESENDE (2018) the presence of hydroxyl groups facilitates the reaction with degradative enzymes.

Finally, if the larva is able to biodegrade the material, the progress of the research will be given in order to isolate and potentialize the enzyme or microorganism contained in the body of Galleria mellonella so that the degradation of this polymer can be carried out on an industrial scale.

6. ANNEXES

ANNEX 1

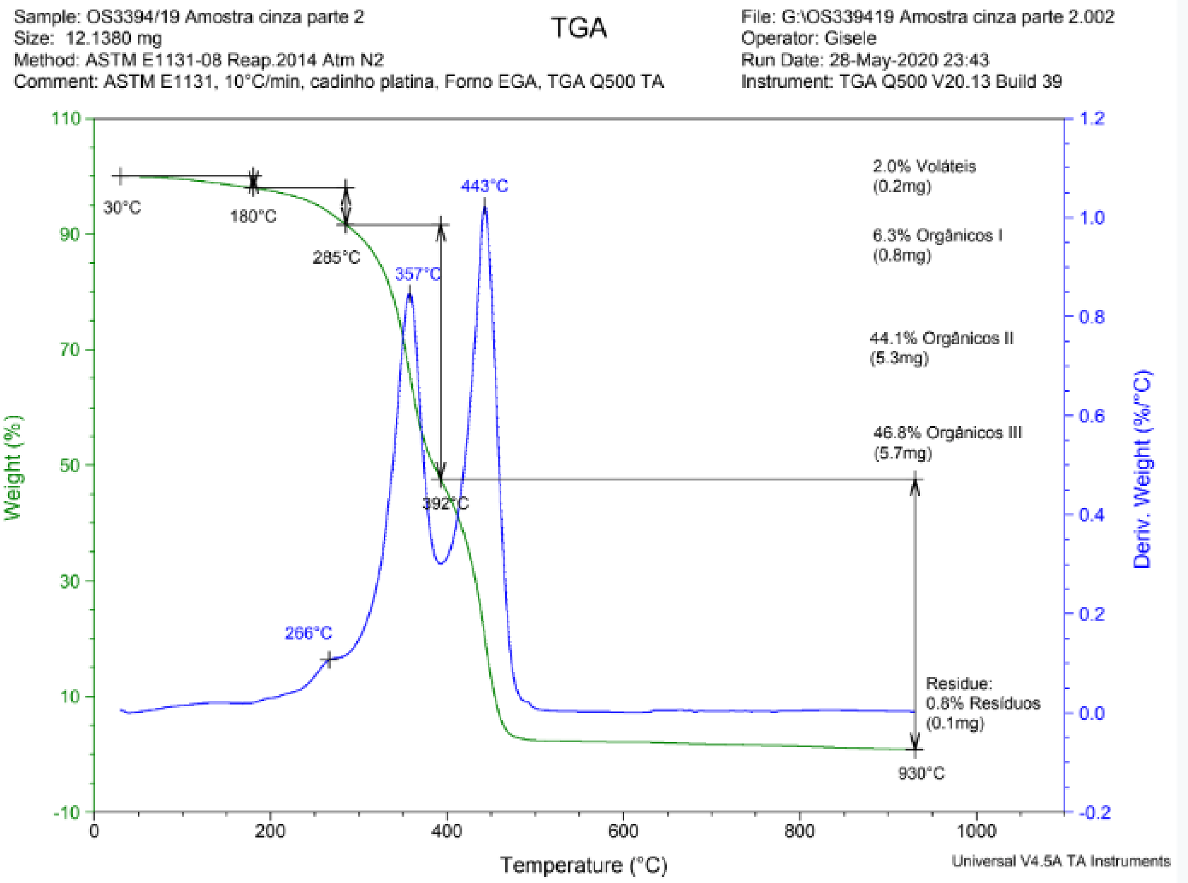
Graph of Total Attenuated Reflectance Spectroscopy (region 3) compared to the reference pattern of Acrylic Resin (in red).



Source: project contributor Gislene Zehetmeyer - 28/05/2020

ANNEX 2

Thermogravimetric Analysis Graph (region 2)



Source: project contributor Gislene Zehetmeyer - 28/05/2020

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【評語】100043

The idea of using moth larvae to digest plastic, especially polyethylene (considered to be one of the most difficult plastics to break down and the bulk of the world's excess plastic waste), is worth encouraging, https://en.wikipedia.org/wiki/Galleria_mellonella. It should be workable to digest photopolymer using Galleria mellonella larvae. The work comes with spectroscopy analysis (Total Attenuated Reflectance Spectroscopy) to check the residue of digesting. It may be interesting to double check with Raman spectroscopy. Also, pin-pointing the breakdown mechanism or where the breakdown takes place will be invaulable. The research has the future objective of isolating the substance into the larvae responsible for degradation so that it can be degraded on industrial scales.