



**UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ**

**FACULTAD DE CIENCIAS QUÍMICAS**

**POSGRADO EN CIENCIAS EN INGENIERÍA QUÍMICA**

**“Evaluación de propiedades fisicoquímicas,  
mecánicas, térmicas y morfológicas de mezclas  
de poli (ácido láctico) y *Chlorella*.”**

**TESIS PARA RECIBIR EL GRADO DE:  
MAESTRA EN CIENCIAS EN INGENIERÍA QUÍMICA**

**PRESENTA**

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**San Luis Potosí, S.L.P.,**

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**UNIVERSIDAD AUTONOMA DE SAN LUIS POTOSI**

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**FACULTY OF CHEMICAL SCIENCES**

**GRADUATE OF CHEMICAL ENGINEERING SCIENCES**

**“Evaluation of physicochemical, mechanical,  
thermal and morphological properties of poly  
(lactic acid) and *Chlorella* mixtures”**

**A THESIS FOR THE DEGREE OF:  
MASTER’S IN CHEMICAL ENGINEERING SCIENCES**

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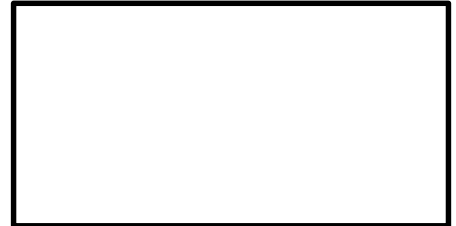
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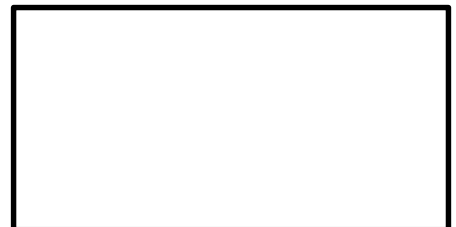
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“Evaluación de propiedades fisicoquímicas, mecánicas, térmicas y morfológicas de mezclas de poli (ácido láctico) y *Chlorella*.” por María Fernanda Méndez Sánchez se distribuye bajo una [Licencia Creative Commons Atribución-NoComercial-SinDerivadas 4.0 Internacional](https://creativecommons.org/licenses/by-nc-nd/4.0/)

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## RESUMEN

En este trabajo de investigación se estudiaron las propiedades fisicoquímicas, térmicas, mecánicas y morfológicas, así como la estabilidad hidrolítica de los compositos obtenidos mediante moldeo por inyección de poliácido láctico y la microalga *Chlorella* a diferentes concentraciones. Se evaluó la influencia de la concentración de la microalga dentro de la matriz polimérica sobre las distintas propiedades estudiadas. Al estudiar la estabilidad hidrolítica de los distintos compositos se encontró que el pH de la solución no influye en la estabilidad de las piezas plásticas. Las propiedades fisicoquímicas, térmicas, mecánicas y morfológicas de los compositos se analizaron con técnicas de termogravimetría (TGA), calorimetría diferencial de barrido (DSC), espectroscopia infrarroja (FT-IR), microscopia óptica (MO), microscopía electrónica de barrido (SEM), pruebas de tracción, Análisis mecánico dinámico (DMA) y determinación del índice de fluidez (MFI). El análisis fisicoquímico y térmico de los compositos mostraron evidencia suficiente para suponer que se lleva a cabo un proceso de extrusión reactiva entre el PLA y la microalga, mediante la interacción de los grupos amino presentes en las proteínas de la *Chlorella*, los cuales reaccionan con los grupos carbonilo presentes en el PLA dando lugar a la formación de iminas y a un probable fenómeno de entrecruzamiento con las cadenas del PLA. En este proceso se ve favorecido por la temperatura de procesamiento del material. Se observó que la primera etapa de pérdida de peso del PLA se logra desplazar por más de 30°C a la mayor concentración de *Chlorella* estudiada. Respecto a las propiedades mecánicas se observó una mejora superior al 250% para la elongación máxima previa a la ruptura para el composito con la menor concentración de *Chlorella* estudiada (3%) respecto a los valores obtenidos para el PLA comercial por lo cual la mezcla PLA-*Chlorella* podría emplearse como alternativa para la fabricación de piezas plásticas mediante moldeo por inyección como una alterativa a polímeros como el polipropileno (PP).

**Palabras clave:** biopolímero; microalga; poli(ácido láctico); *Chlorella*.



## ABSTRACT

In this research work, the physicochemical, thermal, mechanical, and morphological properties of poly(lactic acid) and *Chlorella* microalgae obtained by injection molding at different concentrations were studied, as well as the hydrolytic stability of the composites. It was evaluated the influence of microalgae concentration within the polymeric matrix on the various properties inspected. While studying the hydrolytic stability of the different composites, it was found that the pH of the solution does not influence the stability of the plastic pieces. The physicochemical, thermal, mechanical, and morphological properties of the composites were analyzed using Thermogravimetry (TGA), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FTIR) spectroscopy, optical microscopy (MO), and scanning electron microscopy (SEM), tensile tests, dynamic mechanical analysis (DMA) and determination of the Melt Flow Index (MFI). The physicochemical and thermal analysis of the composites showed sufficient evidence to suppose that a reactive extrusion process is carried out between the PLA and the microalgae, through the interaction of the amino groups present in *Chlorella* proteins, which react with the carbonyl groups present in PLA leading to the formation of imines and to a probable cross-linking phenomenon with the PLA chains. In this process it is favored by the processing temperature of the material. It was observed that the first stage of weight loss of PLA is achieved by moving over 30°C at the highest concentration of *Chlorella* studied. Regarding the mechanical properties, an improvement of more than 250% was observed for the maximum elongation prior to rupture for the composite with the lowest concentration of *Chlorella* studied (3%) compared to the values obtained for the commercial PLA, for which the PLA-*Chlorella* composites could be used as an alternative for the manufacture of plastic parts by injection molding as an alternative to polymers such as polypropylene (PP).

**Keywords:** biopolymer; microalgae; poly (lactic acid); *Chlorella*.

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## CHAPTER 1. GENERAL INTRODUCTION

The global demand for plastic, promoted by the growing use and application of these materials, has increased, generating serious concerns regarding waste management. Because synthetic polymers are resistant to chemical and physical degradation, resulting in a deterioration of environmental quality and a challenge for solid waste treatment plants [1,2]. The main constituents of plastic are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC). All these materials require additives to increase their mechanical properties, impact resistance, and moldability. However, these additives are often toxic and harmful to the environment [3].

In recent years, awareness of the environment has increased, generating multiple investigations focused on developing new materials that are much more environmentally friendly [4–6]. In addition, to this, the recent advances in polymer chemistry and biotechnology have led to the synthesis of new biodegradable polymers that are environmentally friendly and have almost the same applications as petroleum-based polymers. Biodegradable polymers are usually synthesized from renewable resources, such as starch, lignin, cellulose, and proteins, by modifying the natural polymer by adding side substituents, such as hydroxyl and carboxyl amide, and amine groups [1,4,7–9]. The term bioplastic is commonly used for those plastics that represent an improvement for the environment, either because they are obtained from renewable sources (also known as bio-based plastics), or because they are biodegradable (regardless of being bio-based or of petrochemical origin or that are hydrodegradable, oxo-degradable, photodegradable, or degradable by oxidation) or a mixture of these [4,6]. The biodegradation of biodegradable polymers depends on the type of substituent and its location on the polymer chain.

Among the bioplastics of industrial importance are biopolyesters, which poly (lactic acid) (PLA) stands out. The commercial significance of PLA is mainly since it is completely biodegradable and that it can be obtained from renewable resources such as corn, starch, or some microalgae, as well as non-renewable resources. This has driven the interest to use PLA in a wide range of sectors such as the automotive, construction, medical industry, 3D printing, among others. But, unfortunately, PLA is a high crystallinity polymer, which affects its properties, characterizing this material by low elongation at break, low toughness and, consequently, high brittleness [5].

Many investigations have been developed to improve the properties of PLA, mainly focused on three main routes. The first one focuses on searching for new precursors or synthesis mechanisms to obtain PLA with greater stereospecificity, which allows greater control over its properties. Such as the studies carried out by Moon et al. in 2001, they obtained PLLA of high molecular weight through polycondensation catalyzed by a binary system of dehydrated stannous chloride/p-toluenesulfonic acid [10]. The second focuses on studying the copolymerization of lactic acid with different monomers to obtain new polymers with better properties, an example of this route is the study carried out by Zebiri et al. (2021) where they synthesized a triblock, biodegradable and biocompatible copolymer from of PLA and polyurethane [11]. Finally, the third way focuses on the processing and mixing of PLA with different components such as carbon nanotubes [12], calcium carbonate [13], seaweed [14] or microalgae [15].

Microalgae biotechnology has gained considerable importance in recent decades. This is mainly due to the fact that microalgae are considered a promising biological resource for biotechnological applications ranging from biofuels or biopolymers to metabolites of industrial importance thanks to the fact that they are an important biological source for the production of high-value products, among which proteins distinguish, some fatty acids (docosahexaenoic acid, eicosahexaenoic acid,

etc.), pigments ( $\beta$ -carotene, astaxanthin, phycobilin, etc.), anti-aging compounds, skin regeneration products and bioenergy [2,16].

The biomass productivity of microalgae is much higher than that of any other photosynthetic system. In addition, the production of biomass from algae offers several advantages such as higher productivity, higher rates of photosynthetic carbon fixation, efficient nitrogen, and phosphorus removal, when cultivation is done using municipal and industrial wastewater, and without competition with food or crops in agricultural land.

Various studies reveal that there are still multiple scientific and technical problems related to using natural polymers in bioplastics that have been partially solved. Among these, the selection of potential strains to obtain a given polymer for a specific purpose continues to be a problem. In addition, the optimization of mass culture conditions, the potential contamination of the culture, harvesting, and extraction determine the composition and chemical stability of microalgae compounds [2,17]. Therefore, it is crucial to continue researching this field for the above.

In the present study, green microalgae are used at different concentrations as filler material in a polymeric matrix of poly (lactic acid) to improve its thermal, mechanical, and morphological properties without modifying its biodegradability aspect.



## 1.1 Bioplastics as an alternative

Polymers are used in all areas of life, and in recent decades, increasing world population, improved living conditions, and lifestyle have led to a large increase in the consumption of polymers, predominantly polymers petroleum-based [1]. It is estimated that approximately 140 million tons of synthetic polymers are produced annually, which are resistant to chemical and physical degradation. Its stability and non-degradable behavior represent a challenge for solid waste treatment plants because the sanitary landfill is the most common method for the disposal of these, representing between 20% and 25% of urban waste in industrialized countries [2]. Although a great effort has been made to raise awareness about the impact of plastic polymers since the 90s, it has not been enough.

Biopolymers are obtained from different renewable natural sources such as plants, algae, animals, and microorganisms, or they are chemically synthesized from natural products, such as sugars, starch, natural fats, and oils. Most plant-based polymers include pectin, starch, chitosan, xylan, galactoglucomannan, and lignin [2,18]. However, the existing scientific and technological development for biopolymers is still insufficient to replace polymers of petrochemical origin at an industrial level.

All this has promoted the search for much more environmentally friendly alternatives, which range from the development of new polymers to the compatibilization of different biological or bio-based components with petrochemical polymers to improve their biodegradability.

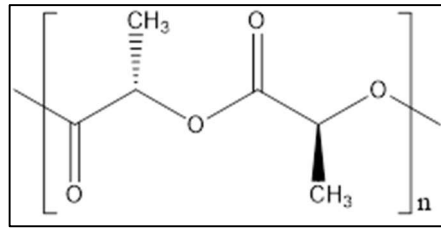
In addition, the use of bioplastics such as polyglycolic acid, poly (lactic acid), polycaprolactone, polyhydroxybutyrate, etc. has been promoted. These are commercially available for biomedical, packing and packaging applications and within

the agricultural, automotive sectors industry, among others. Among all these biopolymers, poly (lactic acid) stands out since it can be obtained from fossil resources, as well as from renewable sources (through microbial fermentation of agricultural by-products). Additionally, when it decomposes, it can degrade to CO<sub>2</sub> and water [19] entirely.

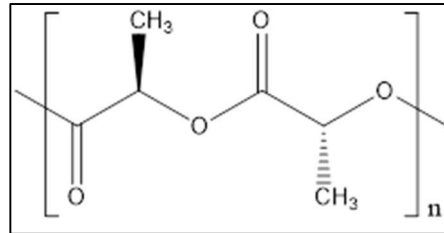
## **1.2 Poly (lactic acid)**

Poly (lactic acid) has existed for decades, but in recent years the interest in it has grown drastically. Thus, numerous and innovative applications have been proposed. PLA is a biodegradable polymer that possesses the potential to substitute existing petroleum-based commodity polymers to help overcome the accumulation of plastic waste. PLA is favored over other biopolymers because it can be mass-produced from petrochemical and agricultural sources, which are renewable, allowing society to reduce its dependence on petrochemicals or alternate using these resources [20].

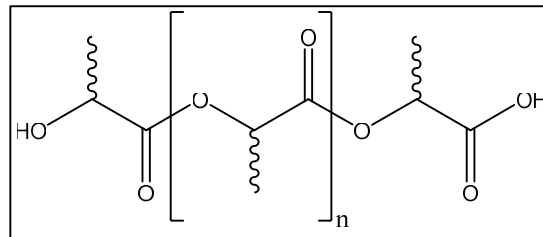
PLA is polylactic acid, a polyester derived from lactic acid produced by the hydrolysis of lactide. Lactic acid is produced in large quantities as a by-product of the fermentation of sugars from corn, sugar cane, and other plants, which makes the commercial production of PLA economically feasible [20]. Depending on the raw materials and the process used to obtain the PLA, this can exist in three stereochemical forms: poly (L-lactic acid) (PLLA) (figure 1.1), poly (D- lactic acid) (PDLA) (figure 1.2), and poly (DL-lactic acid) (PDLLA) (figure 1.3), which are closely related to the percentage of crystallinity, as well as the properties of the polymer [5].



**Figure 1. 1.** Structure of poly (L-lactic acid)



**Figure 1. 2.** Structure of poly (D-lactic acid)



**Figure 1. 3.** Structure of poly (lactic acid)

PLA is considered a brittle material since it has low toughness values and poor elongation at break. In addition, its glass transition temperature is between 60-70 °C, which favors intrinsic brittleness. This fragility is a factor that can be corrected either by copolymerization or by combining with other materials [5,12,19]. Various studies, such as those carried out by Kodal et al. (2019), Sayin et al. (2020), Bulota and Budtova (2015), among others, show that the addition of algae and microalgae to PLA polymeric matrices modifies some of their properties (percentage of crystallinity, biocompatibility, thermal stability, and mechanical resistance) [14,15,21].

Sayin et al. (2020) evaluated the mixture of seaweed in a PLA matrix as an alternative to collagen membranes derived from porcine, in this study they evaluated five types of algae (*Corallina elongata*, *Galaxaura oblongata*, *Cystoseria compressa*, *Sargassum vulgare*, and *Styopodium schimperi*), of which the first three were green algae, and the rest were brown algae. They discovered that all compounds exhibit a similar morphology composed of macroscale pores (which arise due to the difference in surface tension between molten PLA and algae) and microscale defects (due to heating-cooling cycles). Cell proliferation and viability assays demonstrated that all membranes exhibited adequate cell viability. However, the *Sargassum vulgare*-PLA mixtures showed superior proliferation characteristics compared to all others, including the commercial membrane (reference membrane). The authors considered that it could be a feasible option in clinical practice, such as guided tissue regeneration, connective tissue augmentation, and wound regeneration. However, they believed that it is necessary to develop more experimental and clinical studies to clarify the exact characteristics of this composites [21].

Bulota and Budtova (2015) evaluated different biocomposites with poly (lactic acid) as matrix and various algae (red, brown, and green) as fillers by mixing in the molten state, varying the size of the algae (below 50  $\mu\text{m}$  and between 200 and 400  $\mu\text{m}$ ) in concentrations between 2 and 40% by weight of the different algae. This study concluded that there is limited interaction between algae and PLA due to the high concentration of minerals present in the studied algae. This is mitigated by modifying the size and type of algae, obtaining the best result for green algae with a size less than 50  $\mu\text{m}$  [14].

In some cases, plasticizers are used to improve the compatibility between the algae and the polymeric matrix. A study carried out at the University of Malaysia in 2018 made PLA films and used epoxidized palm oil (EPO) with algae powder (not specified) in the ratio 85: 10: 5, respectively. This study showed that the films presented better

properties in terms of tensile strength, flexibility, and dispersion in the PLA matrix [22].

Other studies involving the addition of algae or microalgae in polymeric matrices are carried out by Chiellini et al. (2007) with algae as fillers and prepared poly (vinyl) alcohol (PVA) compounds, by coextrusion, with powder of green algae *Ulva armoricana* and marine algae from the genus *Zostera*. They observe positive results for film-forming, particularly for mechanical characteristics with a limited amount of PVA (40%) attesting for *Ulva* suitability to be introduced in composites (up to 30%). Degradation in the soil of *Ulva*-based composites outlined rapid degradation (over 80% in 100 days), the composite samples underwent different rates of degradation, affected by the different component propensity to degradation [23]. Or the study carried out by Ferrero et al. (2014) of a composition made up of fibers from the oceanic seaweed *Posidonia* and bio-based polyethylene. By using *Posidonia oceanica* as a particle filler for plastics reinforced with natural fibers, they achieved those composites with 30-40% by weight of *Posidonia oceanica* are characterized by superior mechanical performance to HDPE. The water absorption test guarantees dimensional stability of these compounds for outdoor applications. The addition of *Posidonia Oceanica* particles also has a synergistic effect on thermal stability [24], to mention a few.

### **1.3 Chlorella**

*Chlorella* is a genus of single-celled green algae. Its morphology is spherical and has a typical diameter of 2 to 10 micrometers. And it can be cultivated in both fresh and marine water, which may imply that this type of microalgae can have a variable chemical composition [2]. In recent years, *Chlorella* has become popular in the food industry, which has promoted its production under controlled conditions, stabilizing its composition and facilitating its commercial availability, which allows it to expand its field of applications.

A study conducted by D. H. Northcote et al. (1958) reports that the *Chlorella* cell wall contains glucose, galactose, mannose, arabinose, xylose, and rhamnose. As well as some amino acids such as serine, glycine, glutamic acid, threonine, arginine, lysine, histidine, alanine, proline, tyrosine, valine, methionine, phenylalanine, and leucine. The average cell wall composition is estimated to be 27% protein, 9-2% lipid, 15-4%  $\alpha$ -cellulose, a maximum of 31% hemicellulose, and 3.3% glucosamine [17]. Due to the high concentration of proteins in *Chlorella*, and the availability of amino groups in these compounds represent a viable alternative to react with the carbonyl groups present in PLA.

A study carried out by Kodali et al. (2019) reported that the green microalgae of the *Chlorella* genus had nucleating effects. This was demonstrated since the PLA / *Chlorella* compounds presented faster crystallization rates than pure PLA. They prepared films with concentrations of 5 to 20% by weight of *Chlorella* by the method of dissolution in chloroform. The authors reported agglomerations of *Chlorella* at high concentrations of microalgae in the PLA matrix. On the contrary, *Chlorella* was homogeneously distributed within the matrix at low concentrations [11].

## CHAPTER 2. JUSTIFICATION, HYPOTHESIS AND OBJECTIVES

### 2.1 Justification

Poly (lactic acid) (PLA) is an aliphatic polyester that can be obtained from 100% renewable sources, which after being used can be recycled by chemical or physical means. Additionally, it can be completely degraded. These properties made PLA one of the most important industrial bioplastics, however, it still represents an area of scientific and technological interest since PLA has high crystallinity, which results in poor mechanical properties such as low elongation at break, low toughness, and, consequently, high brittleness, making it difficult to use this polymer on a large scale.

As an alternative to improve this polymer's mechanical, thermal, and morphological properties, it is proposed to evaluate binary mixtures of poly (lactic acid) and the microalgae *Chlorella* at different microalgae concentrations. This is since *Chlorella* has a high content of proteins (56%), carbohydrates (17.5%) and lipids (15.5%), which present different functional groups, such as amino, carboxylic and hydroxyl, which can interact and / or react with the carbonyl groups present in PLA. In addition to this, *Chlorella* is a commercially available microalgae, produced under controlled conditions, which results in stable chemical composition, allowing the results of this study to be reproducible.

## 2.2 Hypothesis

*Chlorella* microalgae could act as a filler material as well as a crosslinking agent in a poly(lactic acid) polymeric matrix. The possibility of physical and chemical interactions of the PLA-*Chlorella* system could be due to the presence of amino, carboxylic, and hydroxyl groups in the microalgae with the carbonyl groups present in PLA. The formation of these interactions could be favored by the processing conditions during the injection molding of the composite.

The processing of poly (lactic acid) with different concentrations of *Chlorella* modifies the mechanical, thermal, and morphological properties of PLA, due to the interactions between these materials, giving a crosslinking effect of *Chlorella* on the different polymeric chains of PLA, providing new properties to the polymer.



### **2.3 General objective**

To obtain composites of polylactic acid and Chlorella, using the method of intercalation from the melt by injection molding and characterize the physicochemical, mechanical, thermal, and morphological properties of the composites obtained.

### **2.4 Specific objectives**

- To select the appropriate composition of PLA and Chlorella for the processing of the composites.
- To obtain PLA and PLA-Chlorella plastic parts.
- To determine the physicochemical, mechanical, and thermal properties of PLA-Chlorella composites, as well as the morphological properties.
- To evaluate the short-term hydrolytic stability of composites.
- To determine the mechanism of physical and chemical interaction in PLA-Chlorella composites.
- To determine the potential application of PLA-Chlorella composites.

## CHAPTER 3. MATERIALS AND METHODS

### 3.1 Materials

The poly(lactic acid) (PLA) resin commercial powder was donated from TS Greenova factory and used as the polymeric matrix for biocomposites preparation. The PLA resin composition was 70% PLA, 23% CaCO<sub>3</sub>, and 7% talc, and showed at 190 °C a flow index of 18 g/10 min.

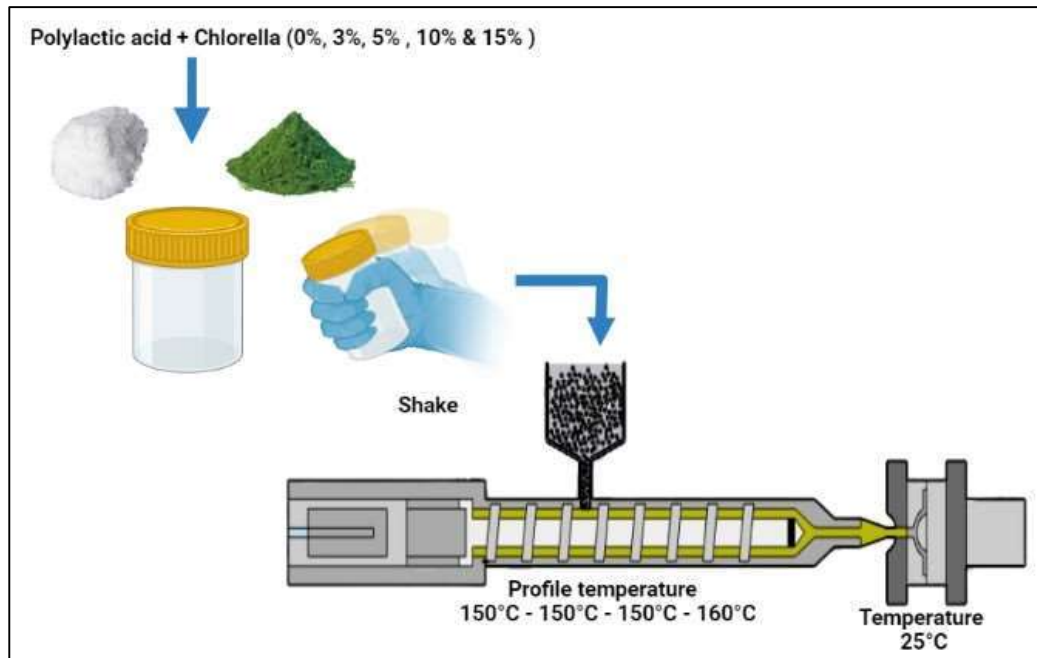
The *Chlorella* (GRASS degree) algae were purchased from SaludVida (For every 100g it contains 57 g of protein, 3.3g of fat-saturated, and 2.9g of dietary fiber).

### 3.2 Fabrication of PLA-*Chlorella* composites

The PLA-*Chlorella* composites were obtained by injection molding in the Nissei injection molding machine NS20. Polymeric mixtures having 0 to 15 wt % *Chlorella*. The PLA and *Chlorella* powder were previously premixed by shaking in a jar. The temperature profile in injection molding was 150 °C in the feed and 160 °C at the nozzle, and the mold temperature was maintained at 25 °C (Figure 3. 1). All samples, including neat PLA and composites, were through the same molding cycle. The samples obtained and their formulations are summarized in Table 3. 1.

**Table 3. 1.** Composition of polyblend composites PLA and *Chlorella*

Sample label	PLA (%W/W)	<i>Chlorella</i> (%W/W)
I-PC00	100	0
I-PC03	97	03
I-PC05	95	05
I-PC10	90	10
I-PC15	85	15



**Figure 3. 1.** Schematic illustration of the injection molding process.

### 3.3 Characterization techniques

#### 3.3.1 Chemical characterization

##### 3.3.1.1 Infrared (IR) spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR spectroscopy) is a molecular characterization technique since its energy (A between 2.5 to 15 pm) excites the vibrational and rotational states of the bonds in the molecules and whose frequency and intensity are related or are a function of the type of bond and the degree polarity of this bonds, giving a specific spectrum of absorption or emission [25]. This technique will be used as part of the characterization of PLA and PLA-*Chlorella* mixtures to observe characteristic bands of the functional groups of interest (C=O, C=N, C-N, etc.).

FTIR spectroscopy was recorded to identify PLA, *Chlorella*, and PLA-*Chlorella* functional groups. A Cary 660 FTIR spectrometer coupled to an attenuated total reflectance (ATR) accessory was used. All spectra were obtained at room temperature with  $4\text{ cm}^{-1}$  resolution and 5000 scans.

### **3.3.2 Thermal characterization**

#### 3.3.2.1 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis is a technique that measures the mass of a sample either isothermally or while it is heated (or cooled) in a defined atmosphere. It is mainly used for the quantitative analysis of products. A typical TGA curve shows the mass loss steps related to the loss of volatile components (moisture, solvents, monomers), polymer decomposition, carbon black combustion, and final residues (ash, fillers) [26]. This method will allow us to study and determine the decomposition temperatures of individual polymers and different binary mixtures. With which the correlation of the microalgae mass vs thermal stability will be established.

Thermogravimetric analyses (TGA) were performed using a TA Instrument®Q500 in a dry nitrogen atmosphere (20 psi). All samples were heated at a scanning speed of  $10\text{ }^{\circ}\text{C}/\text{min}$  from 30 to  $500\text{ }^{\circ}\text{C}$ .

#### 3.3.2.2 Differential Scanning Calorimetry (DSC)

Differential calorimetry analysis is a technique used for thermal transitions. In DSC the sample requires a certain energy to undergo a thermal transition, such as

melting point. This energy difference is recorded as a peak in the DSC scan that indicates the temperature and energy of the transition [27].

Glass transition temperatures were calculated by Differential Scanning Calorimetry (DSC) using a PerkinElmer® DSC 8500 with autosampler under a nitrogen atmosphere. The samples were heated at 10 °C/min from -60 to 190 °C. Values from DSC are reported as the mean  $\pm$  standard deviation from three independent experiments.

### ***3.3.3 Morphological characterization***

#### **3.3.3.1 Optical microscopy**

The optical microscope uses a series of lenses to magnify an image of the surface of a sample, which will allow the observation and surface morphological characterization of polymer composites to be carried out. However, a characteristic microscopic examination requires different levels of observation that, among other aspects, must correspond to different needs in magnification [28].

An optical microscope (Inskam® model 315) was used to obtain the samples structural information, such as texture and microstructure. It was used a 15X magnification.

#### **3.3.3.2 Scanning electron microscopy (SEM)**

The scanning electron microscope uses a beam of electrons to form an enlarged image of the surface of an object, which will allow the observation and surface

morphological characterization of polymer composites [28]. From the interaction between the electron beam and the surface of the sample, signals can be obtained from:

- Secondary electrons (SE), which provide information on the surface morphology of the sample.
- Back-scattered electrons (BSE), which generate images with different brightness depending on the surface chemical composition.

The Scanning Electron Microscopy (Quanta FEG 250 microscope operated at 20 kV) was used to determine the morphology of biocomposites. All samples were prepared by dipping them in liquid nitrogen for 10 minutes with a subsequent fracture. A layer of gold was deposited on the biocomposite surface by sputtering at 20kV to make them conductor. The SEM micrographs were obtained at 100, 500, 1000, and 5000 X magnification.

### ***3.3.4 Mechanical characterization***

#### **3.3.4.1 Tensile test**

It is a characterization technique in which a specimen is stretched, and the load it supports is measured. The correlation between the stress vs. the stress deformation will be established, which allows us to obtain the resistance of a material to a static force [29].

The mechanical properties of the polymers were then determined by tensile tests performed on dog bone-shaped samples. It was used an INSTRON Series IX universal tensile testing machine with a load cell of 1000 N. Previously, a preload of 0.5 N was used to adjust the position of the bone-shaped samples. The extension monitored was given by the separation between the grips. The stress-strain curves were obtained at 2 mm/min speed at room temperature in triplicate.

### **3.3.5 Rheometric characterization**

#### 3.3.5.1 Dynamic mechanical analysis (DMA)

It is an analytical method to determine the viscoelastic properties of materials. The principle used for this method consists in applying an oscillating load to a sample sinusoidally, the sample is also deformed with a sinusoidal function and if the material is deformed in its region of linear viscoelasticity this deformation will be reproducible. With the DMA, the elastic modulus (or storage modulus), the viscous modulus (or the loss modulus) and the damping coefficient (Tan D) will be determined as a function of temperature or time [30].

The dynamic mechanical properties (storage modulus ( $G'$ ), loss modulus ( $G''$ )) of pure PLA and PLA-*Chlorella* composites were studied using a dynamic mechanical analyzer TA Instrument®Q800. The mechanical properties were determined with a 3-point bending test as a function of frequency. The laminates were cut into samples having dimensions of 50 mm × 13 mm × 3 mm. Experiments were carried out at 30°C within a frequency range of 0 –50 Hz.

### 3.3.5.2 Melt flow index

The melt flow index is used to characterize a polymer melt and as a simple and rapid means of quality control. It takes a single-point measurement using specific standard test conditions for each polymer class on a piston-type extruder or extrusion plastometer. The standard procedure for testing the flow rate of thermoplastics using an extrusion plastometer is described in the ASTM D1238 test. During testing, a sample is heated in the barrel and extruded from a short cylindrical die using a weight-actuated piston. The weight of the polymer in grams extruded during the 10 minutes test is the melt flow index (MFI) of the polymer [31].

The melt flow index of PLA and composites was determined using a WANT DW5450A plastometer according to ASTM D1238 procedure B. The measurements were conducted at 190 °C under a 2.16 kg load.

### **3.3.6 Other studies**

#### 3.3.6.1 Hydrolytic stability

The hydrolytic stability of composites at pH 4, 7.4, and 10 was gravimetrically monitored at room temperature. This study was carried out over 1000 hours. As an initial step, the dried samples were weighed and dipped in 5mL of buffered aqueous solution. The influence of pH on the degradation behavior was tested at pH 4 ( $\text{CH}_3\text{COOH}/\text{CH}_3\text{COONa}$ ), pH 7.4 (PBS), and pH 10 ( $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$ ). In all cases, after the samples were dipped for more than a thousand hours under the different conditions, they were lyophilized and weighed again [32].



## **PERSPECTIVES.**

Carry out a detailed characterization of the chemical composition of *Chlorella*.

Evaluate PLA-*Chlorella* composites by nuclear magnetic resonance (NMR) and mass spectrometry (MS) to elucidate and clearly quantify the chemical interactions present between PLA and microalgae.

Obtain PLA-*Chlorella* composites with concentrations less than 3% and evaluate their properties.

Evaluate the degradation kinetics of composites both by composability and by microbial degradation.

Obtain prototypes and compare PLA and *Chlorella* composites in relation to currently used materials.

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