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Comparative Study of Properties of Biopolymers from Corn Starch with Addition of Microalgae *Spirulina platensis*

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Abstract: The discovery of the environmental impacts caused by petroleum-based polymers has led to the use of natural polymers gaining more and more space. Naturally occurring polymers, also known as biopolymers, are chemical compounds produced by living things or raw materials from renewable energy sources. Their main advantage is decomposition, while polymers from fossil and non-renewable energies can take hundreds of years to decompose, biopolymers have significantly shorter life cycles. In this study, a study of the application of the biomass of the microalgae *Spirulina platensis* in biodegradable films with corn starch was conducted, aiming for the development of a functional film with rapid degradability. Approximately 48 biofilms were produced in varying concentrations (w/v), where the visual characteristics of each were observed and the ones that presented the greatest resemblance to conventional plastics were selected, being Trial 4 (T4) and Trial 46 (T46), composed of 2 and 70% v/v of microalgae, respectively. The other tries were discarded due to cracking, high fragility, and very gelatinous or very rigid appearance. The morphological characteristics of T4 and T46 biofilms were analyzed by Scanning Electron Microscopy (SEM) and compared to those of a conventional plastic bag and a commercially available biodegradable plastic bag, where it was possible to prove that the biofilms produced presented good morphological structure. The Fourier Transform Infrared Spectroscopy (FTIR) analysis provided structural information, proving the presence of polyhydroxyalkanoate in the biofilms produced. Two degradability tests were performed with satisfactory results obtained, proving the rapid degradation of the biopolymers produced. It was possible to prove that the biofilms under study present great potential for replacing conventional polymers.

Keywords: Biopolymers, Polyhydroxyalkanoates, Microalgae, Corn Starch

Introduction

For thousands of years, substances that are essential for their existence have been taken from nature, in addition to several other products that help in the comfort and quality of life of mankind. Through the search for these substances, it was realized that there is a wide variety of raw materials and products that can be synthesized from them, such as polymers (Silva, 2011).

The evolution of the study and use of polymers is due to the role industries have played in the applicability of these products. Besides being essential to human life, they are also effective in terms of their durability and flexibility. However, these two qualities have also become a problem over the years, since due to their high durability, their degradation time in the environment after disposal can reach more than 400 years. Moreover, most synthetic polymers are derived from oil, a finite and non-renewable resource, which creates another problem for the environment and the entire ecosystem's raw material chain (Silva, 2011).

In the late twentieth century, the damage caused by polymers in the environment became a concern worldwide. As an alternative to this scenario, biopolymers or biodegradable polymers emerged, which are plastic materials with similar properties to those of conventional polymers, but with a shorter degradation time (de Lima and de Souza, 2011).

Biopolymers derive from raw materials that are totally or partially produced from renewable sources, such as corn, sugar cane, cassava, and microalgae. The main advantage of biopolymers is degradation in the

environment, a process resulting from the action of naturally occurring microorganisms such as algae, fungi, and bacteria (Damodaran *et al*., 2007).

In this study, a study of the application of *Spirulina platensis* microalgae biomass in biodegradable films with corn starch as a gelatinous matrix was carried out, aiming at the development of a functional film with fast degradability.

The addition of the microalgae *Spirulina platensis* to biofilms with corn starch aimed to study different concentrations of both raw materials in order to further decrease the time of degradation of biopolymers. In addition, it aims to replace the main resource, since starch demands a large number of resources for production, such as agricultural land, water, and fertilizers. Microalgae, on the other hand, are easy to cultivate and have a low impact on the food chain, which makes them the best choice for the production of bioplastics (Wang, 2014).

Biopolymers

The use of biopolymers has been growing significantly over the years, with the packaging sector being the largest field of application of this material. In addition, bioplastics are widely used in restoration products, agriculture, or electronic devices (Fabra *et al*., 2018).

The decomposition of the material occurs in its transformation into smaller molecules, causing less environmental impact. Still, the option for bioplastic contributes to the preservation of finite resources such as oil (Damodaran *et al*., 2007).

Another relevant point is that biopolymers apply to the concept of Circular Economy (CE), where the economy is visualized from circularity, in order to reduce the consumption of virgin raw materials and increase the reuse of resources. This concept is based on ecological fundamentals, where there is a shift from the "reduce, reuse, and recycle" paradigm to a deeper and more lasting transformation that aims to reduce the impact of human activities on the environment (Fernández *et al*., 2022).

Aiming to close the production cycle, the circular economy brings both environmental and economic benefits, since the scarcity of natural resources leads to increased product prices (Ferreira *et al*., 2021). There are two groups of materials in the CE concept: Technical materials and biodegradable materials. Technical materials need to be disassembled and recovered, requiring investment in innovation and eco-design. Biodegradable materials, on the other hand, are used and inserted into nature, as in the case of biopolymers (Ferreira *et al*., 2021).

Due to the high quantity of polymers being used in the current economy, the plastic industry stands out in the circular economy scenario, since, to eliminate plastic waste in the environment it is not enough to think only about recycling but to manufacture a polymer that can be inserted in a sustainable way in nature (Ferreira *et al*., 2021).

From this, biopolymers become an alternative, due to the use of renewable sources for their manufacture and their biodegradable potential. Starch-based biofilms have been widely studied in the last decades due to their low price, wide availability, and high purity. However, it is necessary to search for alternative sources of biopolymers that do not compete with food, since the demand for food is only expected to grow over the years (Fabra *et al*., 2018).

In this context, microalgae emerge as a raw material of interest to replace starch and researchers have studied ways of its application in the production of bioplastics. Also, it is worth noting that the use of microalgae in the development of biofilms would increase the sense of circular economy since the resources from the sea would return to it, where they were partially obtained and where they can be degraded and absorbed again (Fabra *et al*., 2018).

Microalgae

Microalgae are microscopic organisms found in aquatic environments and have photosynthetic capabilities. Like plants, they use solar energy, carbon dioxide (CO_2), and water, and release oxygen (O_2). The term "microalgae" refers to algal microorganisms with chlorophyll and other photosynthetic pigments, also encompassing cyanobacteria (Camargo, 2018).

In recent years, microalgae have been the target of interest of scientists due to their versatility to be used in various sectors, such as in renewable energy, the food industry, animal health, human health, and wastewater treatment (Abreu *et al*., 2021). In addition, microalgae present a promising future for the production of biopolymers, due to their high protein content and the small size of their biomass, which allows them to be suitable for conversion into plastic without prior treatment, making the scalable production more economical and reducing waste production (Zeller *et al*., 2013).

These microorganisms have great sustainable potential because their cultivation does not require large areas of land and can occupy locations unsuitable for agriculture, not competing with food production. Another point of interest is that microalgae are able to directly use carbon dioxide (CO_2) to produce biomass, which consequently minimizes the environmental problems caused by CO_2, the main greenhouse gas. Still, in a sustainable way, microalgae can be cultivated in wastewater, where they would serve as treatment in the removal of heavy metals and nutrients and generate biomass, a product of interest for the production of biopolymers (da Silva Braga *et al*., 2019).

There are several species of biopolymer-producing microalgae, such as *Chlorella vulgaris*, *Synechococcus*, *Cyanobium*, *Nostoc*, and *Spirulina platensis* (Martin *et al*., 2014). According to Martin *et al*. (2014), in a study to determine the crude biopolymer productivity of these species, *Nostoc ellipsosporum* and *Spirulina platensis* showed higher biopolymer production.

Fig. 1: *Spirulina platensis* structure the (University of Texas, 2022)

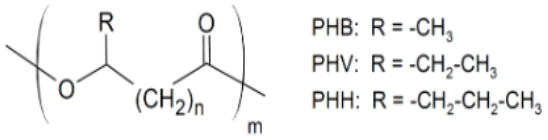


Fig. 2: Molecular structure of polyhydroxyalkanoate (Polymerdatabase, 2015-2022)

As represented in Fig. 1, the microalga *Spirulina platensis* is a filamentous cyanobacterium, blue-green in color, prokaryotic, and can be found in alkaline lakes (Ashby and Houmard, 2006). *Spirulina platensis* is capable of developing in various environments, such as soils, swamps, alkaline lakes, brackish, marine, and fresh waters (Raven *et al*., 2014).

Most of the dry mass of *Spirulina platensis* is formed by proteins, corresponding to approximately 60%, being this percentage varies according to the species and growing conditions. In addition, *Spirulina platensis* has a good amount of pigments, fibers, and minerals such as calcium, iron, phosphorus, copper, magnesium, manganese, potassium, boron, and zinc (Vonshak, 1997).

According to Bennouna (2020), *Spirulina platensis* can be easily cultured in tanks or photobioreactors. Among the most important cultivation parameters are the sources of carbon, nitrogen, and light, the agitation for cell circulation, the configuration of the photobioreactor, the optimal temperature (30-35°C), and the basic pH (Bennouna, 2020).

Cyanobacteria, as photoautotrophic organisms, use light as an energy source to convert CO_2 into biomass. Under nutrient deprivation, most cyanobacteria are able to produce Polyhydroxyalkanoates (PHAs) as energy and intracellular carbon storage compounds (Troschl *et al*., 2017). Under nutrient deprivation, most cyanobacteria are able to produce Polyhydroxyalkanoates (PHA's) as intracellular energy and carbon storage compounds (Troschl *et al*., 2017).

PHA's are aliphatic polyesters with carbon, hydrogen, and oxygen in their molecular composition, whose general formula is shown in Fig. 2. There are three groups of PHA's, which differ in mechanical and thermal properties: Short-chain PHA, medium chain PHA, and long chain PHA. Among the different types, the short chain PHA Polyhydroxybutyrate (PHB) is the most common and the only PHA produced under photoautotrophic conditions so far (Troschl *et al*., 2017).

Due to its properties of biodegradability, thermoplasticity, and biocompatibility with cells and tissues, PHB is a good alternative to common plastics. In cyanobacteria, PHB is found as an energy and carbon storage compound. The most important growth factor for these microorganisms is the absence of nutrients (Troschl *et al*., 2017).

For cyanobacteria, nitrogen limitation is the main trigger for PHB production. Some strains are not able to bind molecular nitrogen and rely on nitrogen in the form of nitrate or ammonium. These strains, because they are unable to synthesize the proteins needed for reproduction, begin to accumulate storage compounds, such as PHB (Troschl *et al*., 2017). Most *Spirulina platensis* species produce PHB only in amounts less than 5% of the cell dry weight. Therefore, increasing this relatively low PHB amount is the main challenge for further research (Troschl *et al*., 2017).

Corn Starch

Starch is a polysaccharide abundantly present in vegetables such as tubers, rhizomes, and grains, and is one of the most promising raw materials for the production of biodegradable polymers due to its low cost and abundant resources. It can be processed into plasticizers under the action of heat. Among the possible applications of starch-based biofilms, it can be highlighted the use in the manufacture of disposable items such as garbage bags, plant pots, temporary fruit covers, cosmetics, paper and textiles, and in pharmaceutical products (Thiré *et al*., 2004).

Its structuring is in granular form, giving its molecules a certain degree of organization, which characterizes it as partially crystalline (Maia, 2016). The molecular structure of starch consists of two different types of glucose polymers: Amylose, which is a linear α-(1,4) D-glucose polymer (Fig. 3) and amylopectin (Fig. 4), analogous to amylose but with 5% α (1,6) branching. The way the amylose and amylopectin are arranged in the granule, results in the formation of more or less dense regions, in a way that the regions where amylopectin is concentrated are denser or crystalline, and its linear portion is responsible for the origin of this crystallization. On the other hand, the amorphous region is formed by chains of amylose and branches of amylopectin (Silva, 2011).

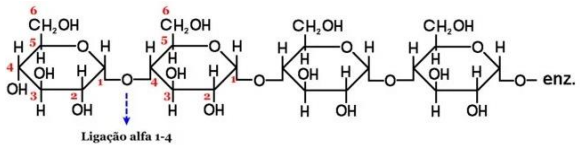


Fig. 3: Amylose molecular structure (Batista, 2011)

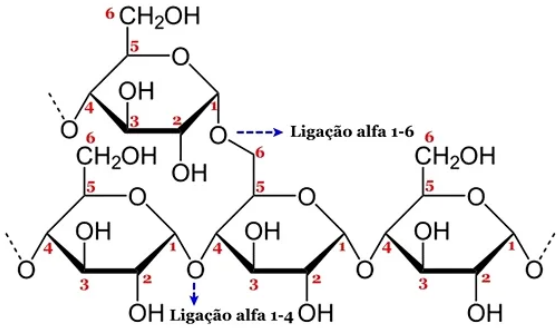


Fig. 4: Molecular structure of amylopectin (Batista, 2011)

Starch granules absorb water and swell when heated in the presence of water. Certain components of starch, especially amylose, leach out and dissolve. With increasing temperature and water absorption, the particles are broken up and the chains are disordered. After cooling, the amylose in the solution undergoes a process called retrogradation, which, if the concentration is high enough, forms a network that converts the solution into a gel. Starch gels are adapted to the matrix filling model, where the filling material is swollen starch granules, and the matrix is formed from the leached starch granules (Cunha, 2017).

The amylose content of common corn starch reaches 28%, being an important factor since it increases the ability of the granules to form a gel after cooking gelatinization a product of interest for the production of biofilms (Damodaran *et al*., 2007; Moura, 2008).

Materials and Methods

Obtainment of Spirulina Platensis Microalgae and Corn Starch

The pure microalgae *Spirulina platensis* from the brand pet store horizonte and the corn starch from the brand Maizena were used, both in powder form and purchased in local stores. All products were used as purchased, without additional purification.

Biofilm Production

The production of biofilms followed the casting technique (Fig. 5), which consists of the solubilization of the macromolecule in a solvent, followed by the application of this filmogenic solution on support and drying in an oven (Krochta *et al*., 1994).

Forty-eight biofilms were produced, most of them with microalgae only, some with cassava starch and *Spirulina*, and others with corn starch and *Spirulina*, where the best combination for the development of biopolymers was obtained. The concentrations (w/v), the plasticizing agent, and the pH were also varied. Table 1 shows all the trials composed by the combination of *Spirulina platensis* microalgae and corn starch, being Trial 4 (T4) and Trial 46 (T46) the only ones with satisfactory results and, therefore, the ones used for the development of this study. In addition, a comparative solution was prepared with corn starch only, using 4g starch, 100 mL distilled water, and 1 mL glycerin (T0).

Visual Analysis

After drying the films, the characteristics of the samples perceptible to the human eye were observed, such as the coloration, fragility, and homogeneity of the films. This was an important step, since brittle films, with high fragility, that presented ruptures, liquid or gelatinous aspects, were discarded.

Scanning Electron Microscopy (SEM)

The Scanning Electron Microscope (SEM) uses an electron beam to provide information about the morphology and identification of chemical elements of a solid sample, quickly (Dedavid *et al*., 2007). If the sample, in this case, the biopolymer, admits a homogeneous form, it is inferred that the materials recognized each other. On the other hand, if the biofilm presents a heterogeneous form and admits a heterogeneous form, the disjunction of the materials occurred (Schaeffer, 2020).

To analyze the microstructural characteristics of the biofilm, it was used the HITACHI SEM, model TM3000 (Fig. 6), available in the Multiuser Advanced Microscopy Laboratory (LMMA) of the Graduate Program in Chemistry of UFVJM-PPGQ/UFVJM.

The samples studied were T4, T46, a sample of biodegradable plastic bag (TA0), and a sample of conventional plastic bag (TB0), in order to analyze the effects caused by inserting quantities of the microalgae in starch biofilms and compare them with plastics available in the market. Both samples, TA0 and TB0, are made of high-density polyethylene-HDPE. The biodegradable one is characterized as oxy-biodegradable, for having the addition of organic metal degrading agents.

The films were fixed on a sample holder (stub) using a tedpella double sided carbon conductive tape. Images were taken using an accelerating voltage of 5 kv, at 100, 500, and 1000× (1 k×) magnification.

Table 1: Biofilm trials with *Spirulina p.* and corn starch

Trial	Composition
2	2 g *Spirulina* powder, 4 g corn starch, 100 mL distilled water, and 1 mL glycerin
4	2 mL filtered *Spirulina*, 4 g corn starch, 98 mL distilled water, and 1 mL glycerin
4 (pH 2)	2 mL filtered *Spirulina*, 4 g corn starch, 98 mL distilled water, 1 mL glycerin, and acetic acid for pH reduction
4 (pH 4)	2 mL filtered *Spirulina*, 4 g corn starch, 98 mL distilled water, 1 mL glycerin, and acetic acid for pH reduction
6	20 mL filtered *Spirulina*, 30 mL slurry water, 0.5 mL polysorbate, 3.5 mL glycerin, 1 g *Spirulina* powder, 1 g cornstarch
27	20 mL filtered *Spirulina*, 3 g corn starch, 4 mL carnauba glycerol
34	1 g *Spirulina* powder, 2 g corn starch, 50 mL distilled water, 17.5 mL sorbitol, and 3.375 mL polysorbate
44	100 mL filtered *Spirulina*, 4 g corn starch, and 1 mL glycerin
45	50 mL filtered *Spirulina*, 4 g corn starch, 50 mL distilled water, and 1 mL glycerin
46	70 mL filtered *Spirulina*, 4 g corn starch, 30 mL distilled water, and 1 mL glycerin
48	70 mL filtered *Spirulina*, 4 g corn starch, 30 mL distilled water, and 1.5 mL glycerin

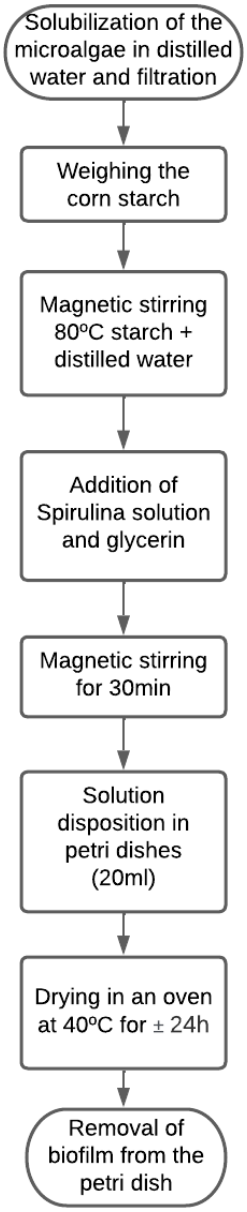


Fig. 5: Flow chart of the biofilm production process

Fig. 6: Scanning electron microscope from Hitachi (Model TM3000)

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR generates standardized spectra that provide structural information. This technique allows the identification and determination of structural characteristics of polymers, especially with regard to functional and bond groups present in the sample. It is the most suitable method for the identification of PHA in the biofilms produced (THERMOFISHER, 2022).

For the analysis, the FTIR used was the Cary 630 model from Agilent (Fig. 7), available in the gas chromatography and FTIR laboratory at UFVJM. After the analysis, the graphic software Origin 8, from Origin Lab, was used to plot the graphs and adjust them.

Fig. 7: FTIR testing machine agilent (model Cary 630)

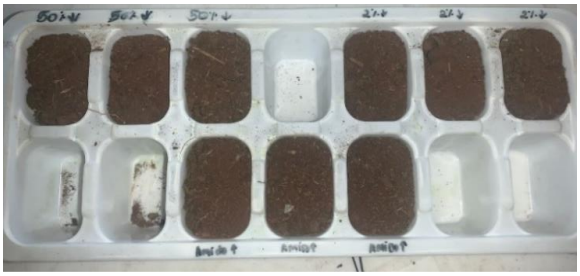


Fig. 8: Samples submitted to the biodegradability test (first system)

Fig. 9: Samples submitted to the biodegradability test (second system)

Biodegradability Test

Among the existing techniques to evaluate the biodegradability potential of a material, the most straightforward is to expose it to the soil. For the test, an adaptation of Astm (2004) was made. The soil used was vegetal earth from gram terra vegeta Brazilian industry. The samples submitted to the test were T0, T4, and T46, with T0 being submitted for the purpose of a comparative analysis of the time of degradation of the biofilm with and without the addition of the microalgae.

Each biofilm was cut into three small pieces, with an area equal to 3.5 cm² (2 × 1.5 cm). Afterward, they were weighed on a mini digital scale and placed in containers containing soil. The samples were kept in a place isolated from light, at a temperature of (21.5±3C°) and relative humidity of (55±5%), and were moistened once a day using 2 mL of water.

Two degradation systems were set up, the first with the sample completely buried, adding a 10 g layer of soil followed by the sample, and finally another 10 g layer of soil (Fig. 8). In the second system, the sample was added only on top of a 10 g layer of soil (Fig. 9). The exposed system aimed to simulate the disposal system in inappropriate places, such as open-air dumps, streets, and beaches. The buried system, on the other hand, represented a correct disposal and destination, as in properly structured landfills.

Results

Visual Analysis

Figures 10-12 show the T0, T4 and T46 biofilms before and after oven drying, respectively.

Figures 13 (T4 with pH 2) and 14 (T44) show two examples of biofilms that did not show satisfactory results and were discarded.

Scanning Electron Microscopy (SEM)

For the analyses in this study, images with 500× magnification were used. Figures 15-18 show the results for samples T4, T46, TA0 and TB0, respectively.

Fourier Transform Infrared Spectroscopy (FTIR)

Figures 19-20 show the Fourier transform infrared spectra of biofilms T4 and T46, respectively.

Biodegradability Test

In this test, the samples were visually analyzed during the 15-day period. Figure 21 (samples T4 and T46) and Fig. 22 (sample T0) show the samples as per the first system, completely buried.

For the second system, with the samples exposed on the ground, the degradation process was recorded with images for two days (Fig. 23), three days (Fig. 24), four days (Fig. 25), eight days (Fig. 26), ten days (Fig. 27), twelve days (Fig. 28), and fifteen days (Fig. 29).

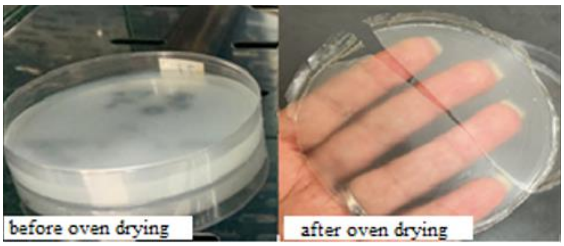


Fig. 10: Biofilm obtained by T0 solution

Fig. 11: Biofilm obtained by solution T4

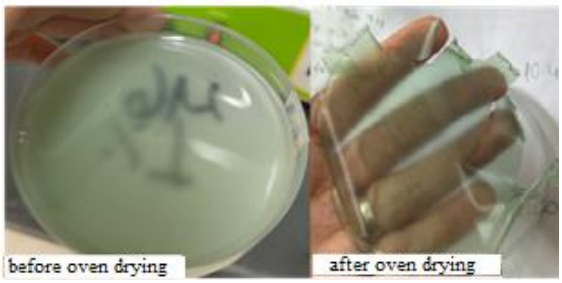


Fig. 12: Biofilm obtained by solution T46

Fig. 13: Trial 4 with pH 2

Fig. 14: Trial 44

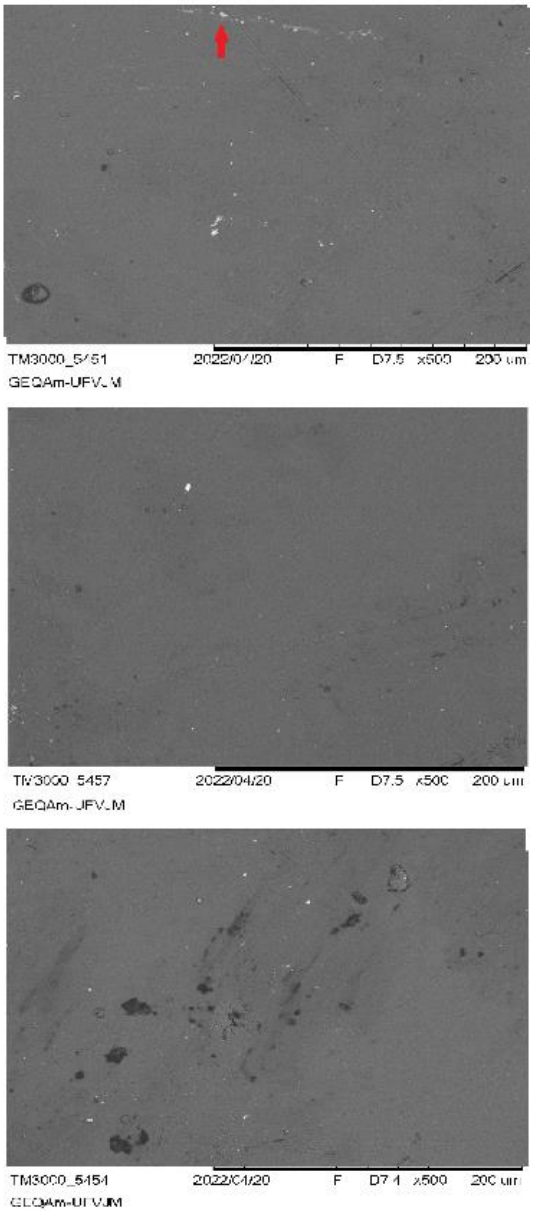
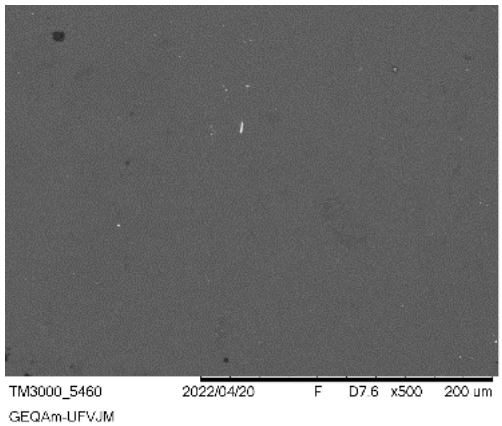


Fig. 15: Result obtained by SEM for sample T4



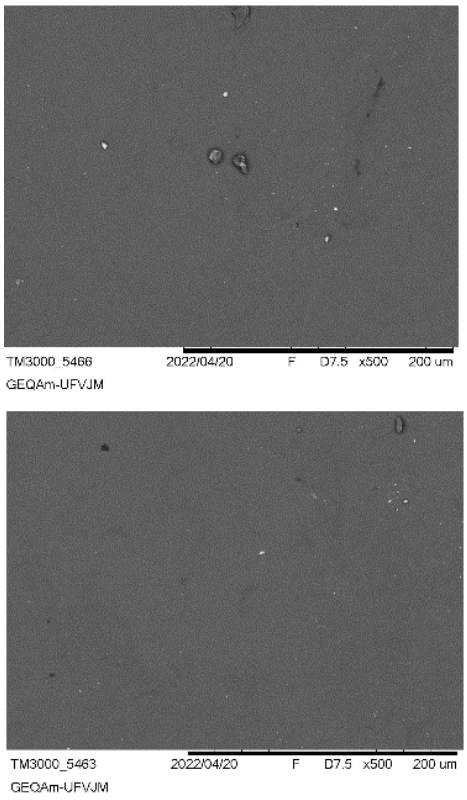
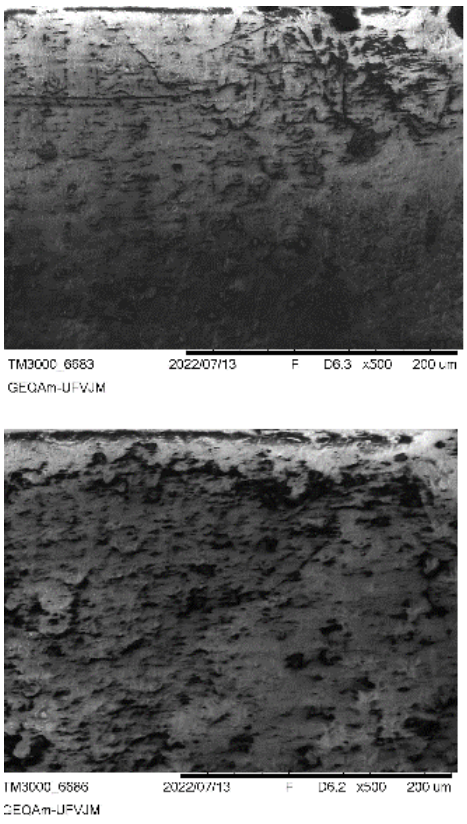


Fig. 16: Result obtained by SEM for sample T46



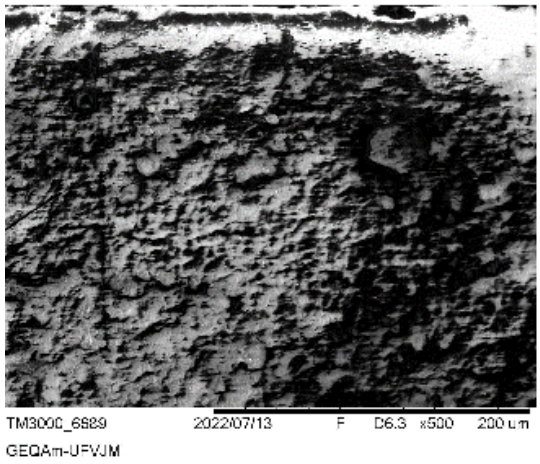
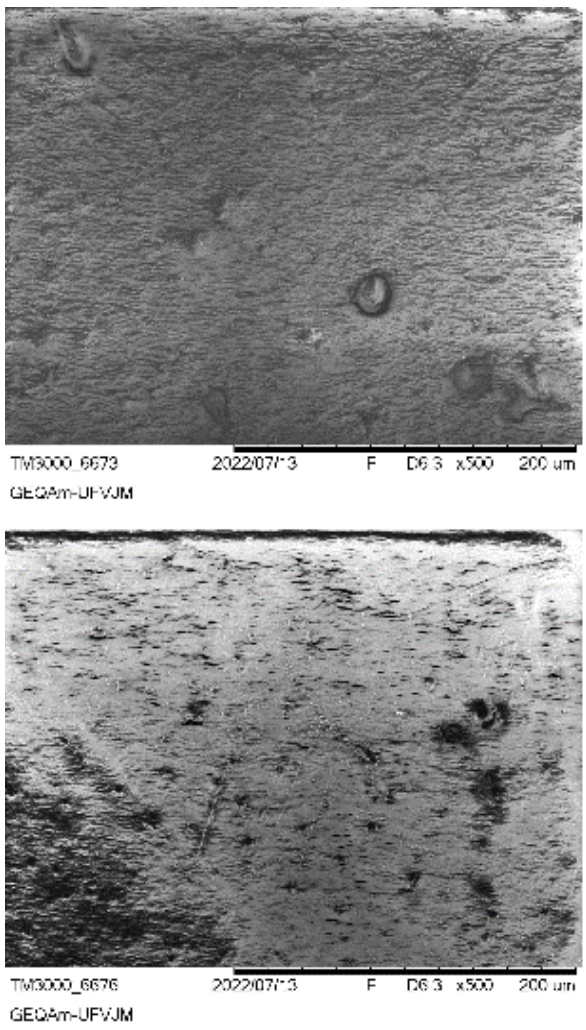


Fig. 17: Result obtained by SEM for sample TA0 (biodegradable bag)



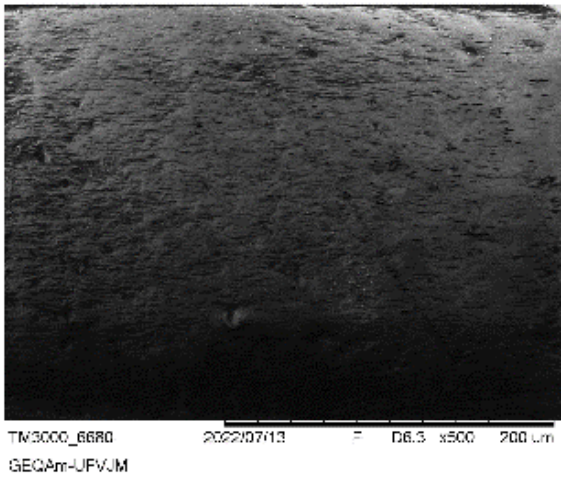


Fig. 18: Result obtained by SEM for sample TB0 (conventional plastic bag)

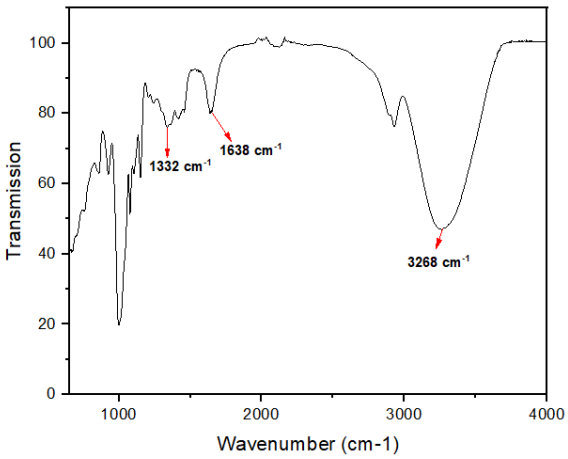


Fig. 19: Infrared spectrum of biofilm T4

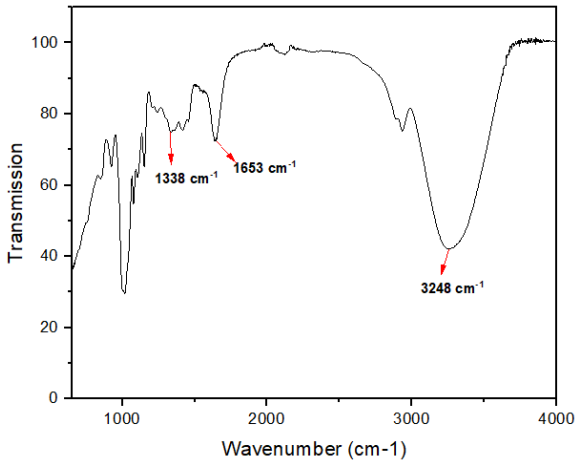


Fig. 20: Infrared spectrum of biofilm T46

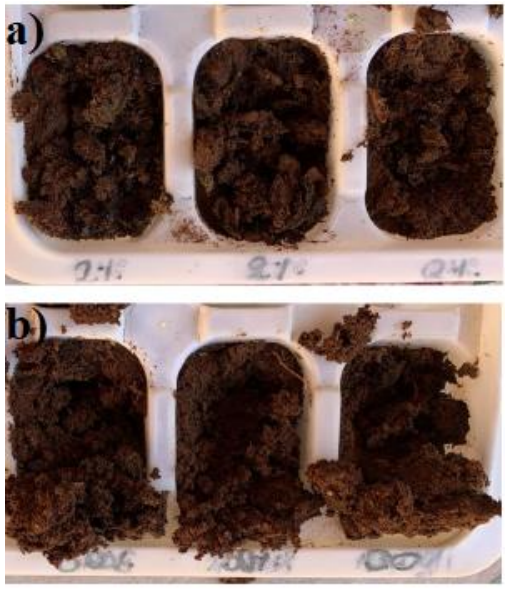


Fig. 21: Samples T4 (a) and T46 (b) on the eighth day of testing

Fig. 22: T0 sample on the eighth day of testing

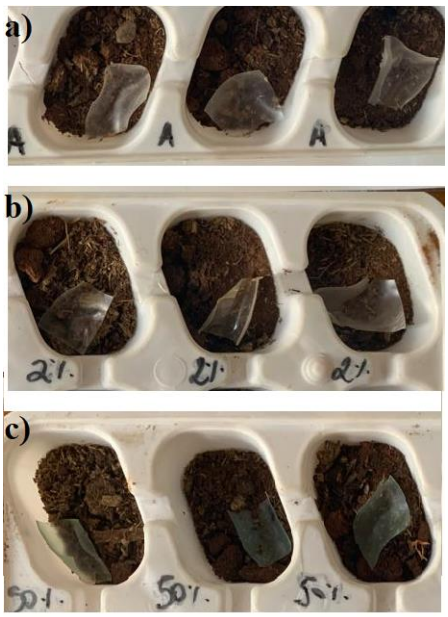


Fig. 23: Samples T0 (a), T4 (b), and T46 (c) on the second day of testing

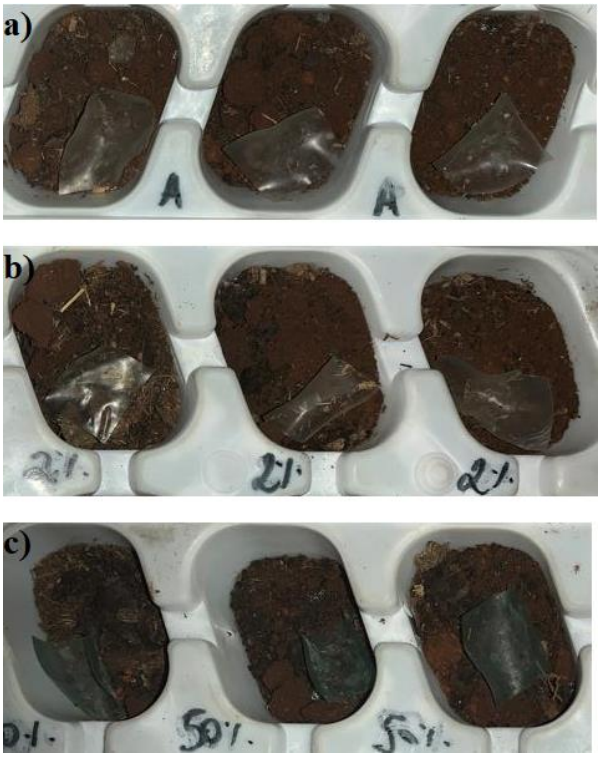


Fig. 24: Samples T0 (a), T4 (b), and T46 (c) on the third day of testing

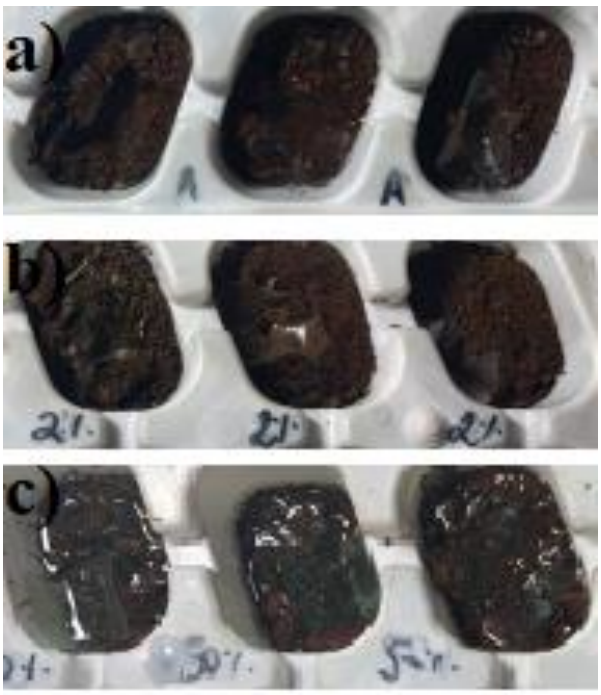


Fig. 25: Samples T0 (a), T4 (b), and T46 (c) on the fourth day of testing

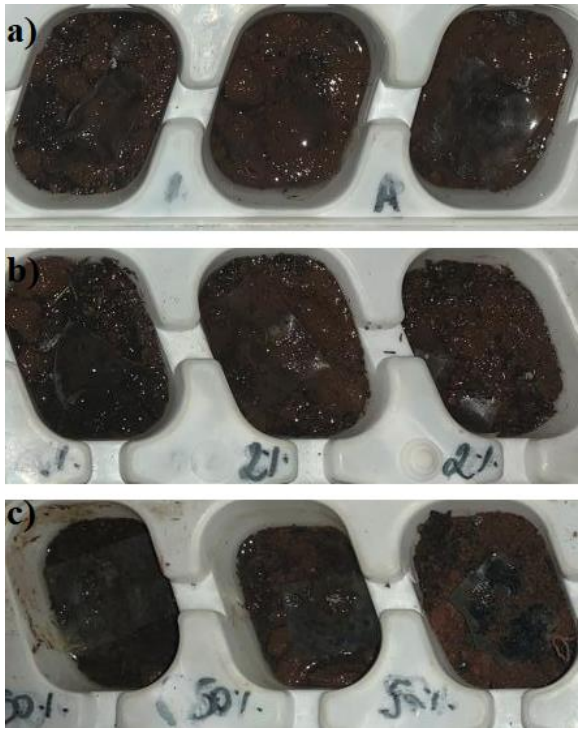


Fig. 26: Samples T0 (a), T4 (b), and T46 (c) on the eighth day of testing

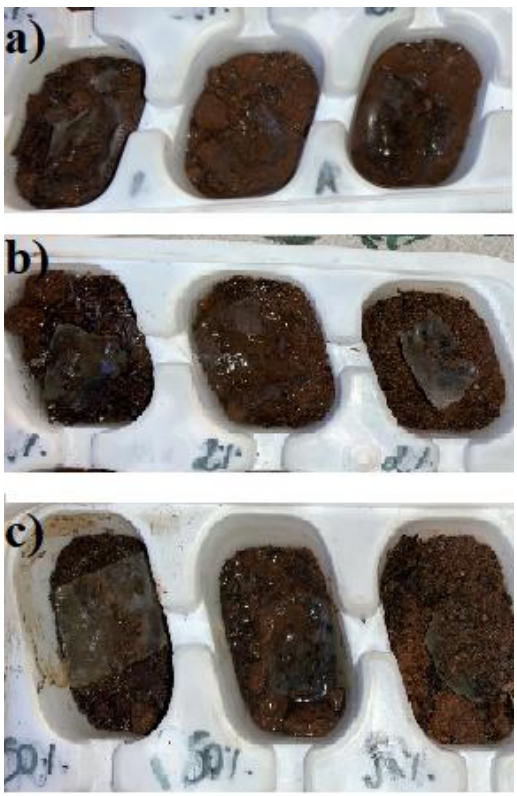


Fig. 27: Samples T0 (a), T4 (b), and T46 (c) on the tenth day of testing

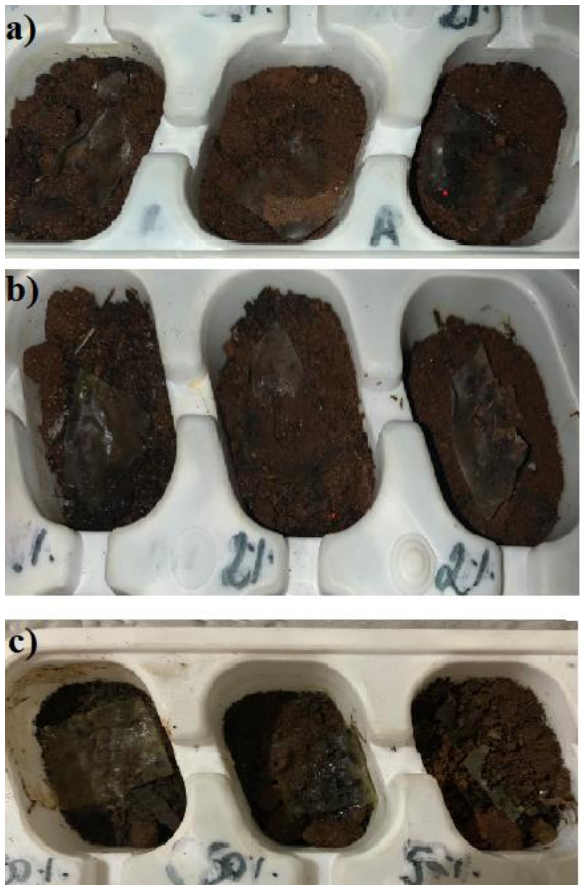


Fig. 28: Samples T0 (a), T4 (b), and T46 (c) on twelfth day of testing

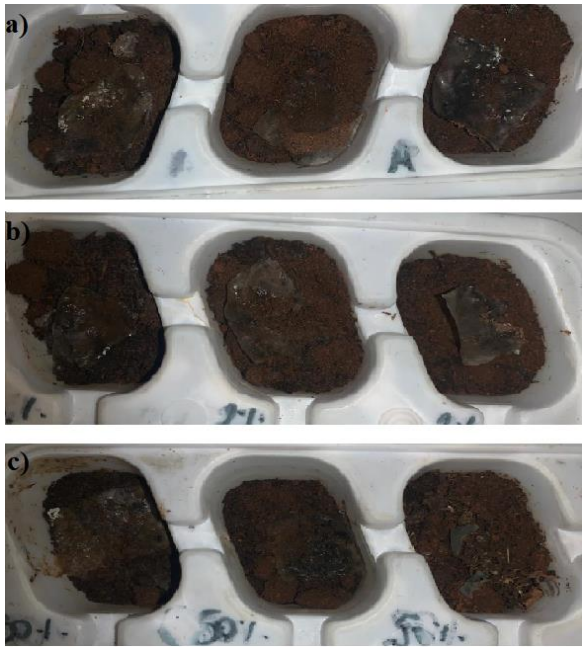


Fig. 29: Samples T0 (a), T4 (b), and T46 (c) on the fifteenth day of testing

Discussion

Visual Analysis

Based on the visual analysis of the biofilms produced, it was observed that the T0 solution (Fig. 10) presented a more gelatinous and dense consistency. Upon drying, its biofilm showed a yellowish color, with a rigid aspect and easier to remove from the petri dish. The T4 solution (Fig. 11), with a 2% concentration of filtered *Spirulina platensis*, was a little more liquid. After drying, a whitish color was noticed in the biofilm, considerable elasticity, and medium difficulty in removing it from the Petri dishes. The T46 solution (Fig. 12), with a concentration of 70% filtered *Spirulina platensis*, showed the most liquid solution, when compared to T0 and T4. Its biofilm presented a greenish color, elastic aspect, and more difficulty to be removed from the Petri dishes.

From the analysis of the coloration of the biofilms, it can be said that the presence of the microalgae attributed to greenish coloration and reduced their transparency, a fact that can be explained due to the scattering of light generated by the presence of the microalgae. For this reason, films containing microalgae can be considered of low transparency, because the transmittance and blurring of the samples are mainly affected by differences in refractive indices between the corn starch matrix and the different compounds that constitute the film structure (Fabra *et al*., 2018).

As for the unsuccessful biofilm attempts, from the analysis of Fig. 13, it was observed that the solution of T4 (pH 2) presented a consistency similar to that of T4 (Fig. 11), however, after drying, there was no biofilm formation. The product presented a rigid and brittle aspect. The solution of T44 (Fig. 14), with a concentration of 100% v/v of filtered *Spirulina platensis* presented a good consistency, being neither too liquid nor too gelatinous. After drying, despite presenting an elastic aspect, its biofilm presented a large number of fissures, which made its use unfeasible.

From these analyses, it can be seen that the higher the concentration of *Spirulina platensis*, the greater the elasticity and fragility of the biofilms. These properties become interesting depending on their commercial application, with characteristics equivalent to those of plastic bags.

Scanning Electron Microscopy (SEM)

From the analysis of the images presented in Figs. 15-18, it was possible to identify the existence of irregularities, pores, cracks, and other microstructural factors that occur in the manufacturing process of the sample and are not visible to the naked eye.

In sample T4 (Fig. 15), small white spots were noticed, which correspond to starch granules that were not well diluted during the preparation of the solution. The darker dots, on the other hand, maybe due to solid particles from

the microalgae that passed during the filtration process. There is also, in the first image on the left, a fissure indicated by the red arrow, which may have been caused by the spatula when removing the films from the petri dish.

For sample T46, which had 70% *Spirulina platensis* microalgae (Fig. 16), one could also see white spots that analogous to sample T4, correspond to poor starch solubilization. However, it can be seen that there are fewer dark spots, which indicates better filtration and possible interaction of the materials. Furthermore, it can be inferred that the microalgae had its cell walls modified after the solubilization process of the films (Fabra *et al*., 2018).

When observing samples TA0 (Fig. 17) and TB0 (Fig. 18), it can be seen that there is the presence of roughness, unevenness, fissures, and larger pores when compared to samples T4 and T46, indicating a more heterogeneous surface. However, they do not present any lumps of poorly solubilized components.

In general, it is noticeable that the corn starch granules solubilized better in a higher concentration of microalgae, inferring an affinity between the compounds. With this, the SEM tests showed that the higher the concentration of *Spirulina platensis*, the greater the homogeneity of the matrix.

It is important to note that more homogeneous matrices result in more resistant bioplastics and, consequently, better applications (Maniglia *et al*., 2017). Finally, it can be seen that the samples produced presented good morphological structure, a favorable result that indicates a considerable alternative for implementation in the market.

Fourier Transform Infrared Spectroscopy (FTIR)

In order to prove the presence of PHA biopolymer in biofilms produced from the microalgae *Spirulina Platensis* and corn starch, the samples were submitted to FTIR analysis. Thus, it was possible to determine the characteristic functional groups of PHA, whose molecular structure is shown in Fig. 2.

From the analysis of the spectra presented in Figs. 19-20, it can be observed that both spectra (T4 and T46) presented bands characteristic of functional groups present in PHA (Colthup *et al*., 1990).

According to Colthup *et al*. (1990), the bands 1638 cm^{-1} (Fig. 19) and 1653 cm^{-1} (Fig. 20) are characteristic of the carbonyl group (C = O), typical of esters, which can range from 1550 to 1900 cm^{-1}. The bands 1332 cm^{-1} (Fig. 19), 1338 cm^{-1} (Fig. 20) and the neighboring bands represent the CH_3 and CH_2 bonds, which vary from 1250-1500 cm^{-1}, also present in PHA's. The bands 3268 cm^{-1} (Fig. 19) and 3248 cm^{-1} (Fig. 20) are characteristic of the hydroxyl group (OH), which can vary from 3100 to 3700 cm^{-1} (Colthup *et al*., 1990).

The band readings are in agreement with the results found in the works of Shah (2012), Shamala *et al*. (2009); Alarfaj *et al*. (2015).

Biodegradability Test

For the samples that were completely buried, it was observed that the degradation process happened faster, with the samples with 2% *Spirulina platensis* solution (T4) and 70% *Spirulina platensis* solution (T46) no longer existing on the eighth day (Fig. 21). The composition sample with only the starch (T0) also degraded rapidly. However, by day 8 there were still small pieces of biofilm (Fig. 22).

For the samples exposed to the soil, the degradation process occurred at a slower rate, where total degradation occurred on the fifteenth day. On the second day of testing (Fig. 23), it was noticed that the samples already presented a deformation and a drier aspect. This process became more noticeable on the third day (Fig. 24). Throughout the fourth day (Fig. 25), it was observed that after the humidification of the system, the samples became wetter and less stiff, indicating a more evident degradation process. On the eighth day (Fig. 26) and on the tenth day (Fig. 27), the samples began to lose color, presenting dark and much wetter spots than on the previous days. By the twelfth day (Fig. 28) it was possible to notice that sample T46 (Fig. 28) was already smaller in size than the other T46 samples and samples T0 and T4. When the time of 15 days was completed (Fig. 29), it was noticed that the degradation process happened faster in the films of samples T4 and T46, with T46 showing a greater loss of characteristics.

Based on the results obtained, it can be inferred that the films are characterized as biodegradable. Samples T4 and T46, both with the addition of the microalgae *Spirulina platensis*, have a faster degradation process than sample T0 and, all have a much faster process than conventional plastic bags, which can take about 20 years to decompose. This time difference is explained by the presence of natural molecules in biofilms, such as proteins and polysaccharides (Santos, 2020).

Conclusion

Based on the literature review and the experiments performed, it is possible to prove that the microalgae *Spirulina platensis* and corn starch are raw materials with great potential for the production of biopolymers. After several attempts with variations of pH, solvent concentrations, microalgae, and corn starch to produce the biofilms, it can be seen that the gelatinization capacity of corn starch favors their formation. With regard to *Spirulina platensis*, the higher its concentration, the greater the elasticity and fragility of the biofilms. Through visual analysis of the films produced, it was noticed that biofilms T4 and T46 presented similarity and compatibility for use as plastic films and plastic bags, respectively.

With the SEM analysis, it can be seen that the samples produced presented good morphological structure, with less roughness, cracks, bubbles, and unevenness than the conventional plastic bag and biodegradable plastic bag samples, a favorable result that indicates a considerable alternative for implementation in the market. From the FTIR technique, it was possible to prove the presence of polyhydroxyalkanoate in the biofilms produced.

The biodegradability test was favorable for samples T4 and T46, which had the shortest time for degradation (8 days for the buried system and 15 days for the exposed system). On the other hand, the biofilm composed only with corn starch (T0), at this same time, presented a degradation in a good part of the material, but it had not degraded completely as samples T4 and T46.

From the results obtained, it can be concluded that the combination of corn starch and *Spirulina platensis* for the production of biopolymers is very promising since the addition of *Spirulina platensis* generated elasticity to the biofilm and enhanced its degradability capacity.

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Author's Contributions

Júllia Koury Abreu and Loraynne Coelho Pereira: Conception and design, and/or acquisition of data, and/or analysis and interpretation of data; contribute in drafted the article or reviewed it critically for significant intellectual content.

José Izaquiel Santos da Silva: Conception and design, and/or acquisition of data, and/or analysis and interpretation of data; contribute in drafting the article or reviewing it critically for significant intellectual content; and give final approval of the version to be submitted and any revised version.

Ethics

This study and the ethical aspect were approved by the authors and collaborators of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM). Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM).

References

Abreu, F. C., Mota, E. A., Pereira, R. V., Oliveira, V. F., Costa, M. P., Gomes, M. D. S., ... & Guerra-Sá, R. (2021). Differential expression profiles of miRNAs and their putative targets in Schistosoma mansoni during its life cycle. *Memórias do Instituto Oswaldo Cruz, 116*. https://www.scielo.br/j/mioc/a/TGBrnLJ46G9Fh3nQrNWXRMz/

Alarfaj, A. A., Arshad, M., Sholkamy, E. N., & Munusamy, M. A. (2015). Extraction and characterization of polyhydroxybutyrates (PHB) from Bacillus thuringiensis KSADL127 isolated from mangrove environments of Saudi Arabia. *Brazilian Archives of Biology and Technology*, *58*, 781-788.

Ashby, M. K., & Houmard, J. (2006). Cyanobacterial Two-Component Proteins: Structure, Diversity, Distribution, andEvolution. *Microbiology and Molecular Biology Reviews*, *70*(2), 472-509. https://doi.org/10.1128/MMBR.00046-05

Astm, G. (2004). Standard practice for calculation of corrosion rates and related information from electrochemical measurements. *G102-89*.

Batista, C., (2011). Starch. AllMatter. https://www.todamateria.com.br/amido/

Bennouna, C., (2020). How to grow *chlorella*? https://www.synoxis-algae.com/como-cultivar-a-chlorella/

da Silva Braga, V., Moreira, J. B., Costa, J. A. V., & de Morais, M. G. (2019). Potential of Chlorella fusca LEB 111 cultivated with thermoelectric fly ashes, carbon dioxide and reduced supply of nitrogen to produce macromolecules. *Bioresource Technology*, *277*, 55-61. https://doi.org/10.1016/j.biortech.2019.01.035

Camargo, L. S. F., (2018). Microalgae?. Science enthusiasts guide. Federal University of ABC. https://gec.proec.ufabc.edu.br/o-que-que-a-ciencia-tem/microalgas

Colthup, N. B., Daly, L. H., & Wiberley, S. E. (1990). Vibrational and rotational spectra. *Introduction to Infrared and RAMAN Spectroscopy*, 1-73.

Cunha, G. (2017). Starch-based biofilms incorporated with ethanolic propolis extract. Federal Institute of Education, Science and Technology of Goiás. https://sistemas.ifgoiano.edu.br/sgcursos/uploads/anexos_11/2017-10-19-09-50-32Disserta%C3%A7%C3%A3o%20Giana.pdf

Damodaran, S., Parkin, K. L., & Fennema, O. R. (Eds.). (2007). *Fennema's Food Chemistry*. CRC press. https://www.taylorfrancis.com/chapters/edit/10.1201/9781420020526-5/introduction-food-chemistry-owen-fennema-srinivasan-damodaran-kirk-parkin

Dedavid, B. A., Gomes, C. I., & Machado, G. (2007). *MICROSCOPIA Eletrônica de varredura: Aplicações e preparação de amostras: Materiais Poliméricos, Metálicos e Semicondutores*. EdiPUCRS.

Fabra, M. J., Falcó, I., Randazzo, W., Sánchez, G., & López-Rubio, A. (2018). Antiviral and antioxidant properties of active alginate edible films containing phenolic extracts. *Food Hydrocolloids*, *81*, 96-103. https://doi.org/10.1016/j.foodhyd.2018.02.026

Fernández, N., Gaggino, R., Kreiker, J., & Positieri, M. J. (2022). Revalorización de residuos de cartón y papel para la producción de biopolímeros dentro del paradigma de economía circular con aplicación en el campo del hábitat. *Brazilian Journal of Animal and Environmental Research*, *5*(2), 2362-2369. https://doi.org/10.34188/bjaerv5n2-076

Ferreira, L., Monaro, D. L. G., & de Oliveira Plens, A. C. (2021). A importância da economia circular para produtos feitos à base de polímero: Uma análise de conteúdo. *Brazilian Journal of Business*, *3*(1), 33-48. https://doi.org/10.34140/bjbv3n1-003

Krochta, J. M., Baldwin, E. A., Nispero-Carriedo, M. O., (1994). Edible Coating and Films to Improve Food Quality. 10^{th}Ed. Technomic Publishing Company. ISBN-10:15-667-61131. https://www.researchgate.net/publication/284773714_Edible_Coatings_for_Lightly_Processed_Fruits_and_Vegetables

de Lima, R. M. F., & de Souza, V. V. (2011). Polímeros Biodegradáveis: Aplicação na agricultura e sua utilização como alternativa para a proteção ambiental. *Revista Agrogeoambiental*. https://doi.org/10.18406/2316-1817v3n12011303

Maniglia, B. C., Tessaro, L., Lucas, A. A., & Tapia-Blácido, D. R. (2017). Bioactive films based on babassu mesocarp flour and starch. *Food Hydrocolloids*, *70*, 383-391. https://doi.org/10.1016/j.foodhyd.2017.04.022

Martin, R. G., Nixon, C., Armitage, P. J., Lubow, S. H., & Price, D. J. (2014). Giant Outbursts in Be/X-Ray Binaries. *The Astrophysical Journal Letters*, *790*(2), L34. 10.1088/2041-8205/790/2/L34

Moura, W. D. S. (2008). Extração e caracterização do amido do Hedychium coronarium e elaboração de filmes biodegradáveis. http://www.bdtd.ueg.br/handle/tede/609

Maia, N. V. L. P. (2016). Produção e caracterização de blendas poliméricas com poli (3-hidroxibutirato)) (PHB) e amido de milho para produção de plastico biodegradável. https://repositorio.ufsc.br/handle/123456789/172365

Polymerdatabase. (2015-2022). Polyhydroxyalkanoates (PHAS).

Raven, P. H., Eichhorn, S. E., & Evert, R. F. (2014). Biology of plants. 8ª Edição. https://edisciplinas.usp.br/mod/folder/view.php?id=2913607

Santos, B., (2020). Biodegradability of films made from polymer and fenugreek essential oil. State University of São Paulo. https://repositorio.unesp.br/bitstream/handle/11449/204387/santos_b_me_ilha_sub.pdf?sequence=5&isAllowed=y

Schaeffer, D., (2020). Obtention and Characterization of Biopolymers from Cassava Starch and Corn Starch. University of Taquari Valley. https://www.univates.br/bdu/bitstream/10737/2858/1/2020DaianeSchaeffer.pdf

Shah, K. (2012). FTIR analysis of polyhydroxyalkanoates by a locally isolated novel Bacillus sp. AS 3-2 from soil of Kadi region, North Gujarat, India. *Journal of Biochemical Technology*, *3*(4), 380-383. https://www.researchgate.net/publication/236628211

Shamala, T. R., Divyashree, M. S., Davis, R., Kumari, K. L., Vijayendra, S. V. N., & Raj, B. (2009). Production and characterization of bacterial polyhydroxyalkanoate copolymers and evaluation of their blends by fourier transform infrared spectroscopy and scanning electron microscopy. *Indian Journal of Microbiology*, *49*(3), 251. https://doi.org/10.1007/s12088-009-0031-z

Silva, E.M., (2011). Production and characterization of biodegradable pinhão starch films. Federal University of Rio Grande do Sul. https://www.lume.ufrgs.br/bitstream/handle/10183/38562/000823833.pdf?sequence=1

THERMOFISHER. (2022). FTIR spectrometers and FTIR microscopes. https://www.thermofisher.com/br/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fourier-transform-infrared-spectroscopy.html. Accessed on March, 2022.

Thiré, R. M. S. M., Simão, R. A., Araújo, P. J. G., Achete, C. A., & Andrade, C. T. (2004). Reduction of the hydrophilicity of starch-based biodegradable films through plasma polymerization. *Polymers: Science and Technology*. *14*, 1. https://revistapolimeros.org.br/journal/polimeros/article/doi/10.1590/S0104-14282004000100015

Troschl, C., Meixner, K., & Drosg, B. (2017). Cyanobacterial PHA production-review of recent advances and a summary of three years' working experience running a pilot plant. *Bioengineering*, *4*(2), 26. https://doi.org/10.3390/bioengineering4020026

University of Texas. (2022). UTEX LB 2340 *Spirulina platensis*. https://utex.org/products/utex-lb-2340?variant=30992067067994. Accessed on April, 2022

Vonshak, A. (Ed.). (1997). *Spirulina platensis arthrospira: Physiology, Cell-Biology and Biotechnology*. CRC press.

Wang, K. (2014). *Bio-plastic potential of spirulina microalgae* (Doctoral dissertation, University of Georgia). https://getd.libs.uga.edu/pdfs/wang_kun_201405_ms.pdf

Zeller, M. A., Hunt, R., Jones, A., & Sharma, S. (2013). Bioplastics and their thermoplastic blends from *Spirulina* and Chlorella microalgae. *Journal of Applied Polymer Science*, *130*(5), 3263-3275. https://doi.org/10.1002/app.39559