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Extraction of poly(3-hydroxybutyrate) from *Spirulina* LEB 18 for developing nanofibers

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Abstract

The objective of this study was to extract poly(3-hydroxybutyrate) (PHB) from the microalgal biomass of *Spirulina* LEB 18 for the development of nanofibers by electrospinning method. Different extraction methods were tested. The maximum yield obtained was 30.1 ± 2%. It was possible to produce nanofibers with diameters between 826 ± 188 nm and 1,675 ± 194 nm. An increase in the nanofiber diameter occurred when a flow rate of 4.8 μL min^{-1} and a capillary diameter of 0.90 mm were used. The nanofibers produced had up to 34.4% of biomass additives, i.e., non-PHB materials. This can be advantageous, because it enables the conservation of microalgal biomass compounds with bioactive functions.

Keywords: *biomass, electrospinning, nanofibers, PHB, Spirulina.*

1. Introduction

Microalgae are photosynthetic organisms with relatively simple requirements for growth when compared with other biomass sources. The composition and rates of photosynthesis and growth of these microorganisms are highly dependent on culture conditions; if these conditions are manipulated, metabolites of interest can be produced[1]. Polyhydroxyalkanoates (PHAs) are biopolymers that are produced and accumulated by microorganisms as energy reserves, and they can be synthesized by microalgae. One PHA that has attracted international scientific, technological and industrial interest is poly(3-hydroxybutyrate) (PHB), due to the fact that it is thermoplastic, biodegradable and biocompatible with cells and tissues[2,3].

Several PHA extraction processes may be used; however, it is important to develop efficient methods that lower production costs[3]. The processes that are most commonly used are based on extraction with trichloromethane (sometimes pre-treated with acetone), followed by precipitation with diethyl ether or methanol[3,4]. Sodium hypochlorite, methylene chloride, dichloroethane and propylene carbonate are also used in extraction processes[3].

Due to its biodegradability and biocompatibility with cells and tissues, PHB has a strong potential for use in the development of nano or microfibers in the fields of food and medicine. Sensors can be placed in packaging to detect pathogens in foods that change the color of the packaging in order to alert the consumer if there is a problem, or to release preservatives if the food begins to deteriorate[5].

Nanofiber technology can be used to incorporate bioactive additives such as probiotics, prebiotics, antioxidants or vitamins in packaging. These additives are released at the time of consumption. Some functional components are not compatible with food: they may produce unwanted flavors and odors, or modify the texture of food, so it is better to incorporate them in the packaging and release them at the time of consumption, rather than adding them to the food itself during processing[5].

Electrospinning is carried out by applying high voltage to a polymer solution in a process that results in nanofiber formation and lengthening due to electrostatic repulsion. The polymer solution is fed at a constant flow rated through a capillary charged with a high tension. When the electric field attains enough energy to overcome surface tension at the tip of the capillary a 'Taylor Cone' forms and the nanofiber are deposited in a collector where the solvent evaporates and the nanofibers collect[6-8].

The objective of this study was to extract poly(3-hydroxybutyrate) from the microalgal biomass of *Spirulina* LEB 18 for the development of nanofibers.

2. Materials and Methods

2.1 Production of the Spirulina LEB 18 (Arthrospira) microalgal biomass

The microalga used in this study was *Spirulina* LEB 18, which was isolated from the Mangueira Lagoon.[9] Zarrouk culture medium at 20% (v/v)[10] supplemented with Mangueira Lagoon water was used during the microalgal development.

The cultures were prepared at a pilot plant to produce *Spirulina* LEB 18, on the edge of Mangueira Lagoon (33º 30' 13'' S and 53º 08' 59'' W). The unit consisted of three 10,000 L raceway-type bioreactors and one 1,000 L raceway-type tank. The reactors were coated with fiberglass and covered with transparent plastic film. The cultures were agitated by rotating blades at 18 rpm[11].

Every 72 h, the microalgal biomass was harvested with the aid of a 200 µm filter. The filtrate was returned to the tanks, and nutrients were used until they were exhausted. The filtrate was concentrated in a hydraulic press, and the biomass was extruded. After extrusion, the biomass was dried at 50 °C for 5 hours in a tray dryer, vacuum packed and stored[11]. Extraction tests were carried out using this biomass.

2.2 Extraction of PHB using 4% sodium hypochlorite

Sodium hypochlorite 10% (v/v) was diluted and the final concentration was 4% (v/v). The microalgal biomass was mixed with 4% sodium hypochlorite and agitated for 20 min, and then centrifuged to separate the dead cells. The process was repeated and it was then rinsed twice with distilled water, followed by centrifugation and rinsing with acetone to facilitate drying. The PHB was stored at 25 ° C for 72 hours for evaporation of water[4] (Sample PHB1).

2.3 Extraction of PHB using trichloromethane

PHB was extracted from the dry microalgal biomass using trichloromethane at 65 °C for 4 h. The biomass containing trichloromethane was filtered to remove ruptured cells. The trichloromethane was evaporated and PHB was precipitated with methanol. PHB was kept at 25 °C for 72 hours for evaporation of water (Sample PHB2).

2.4 Extraction of PHB with trichloromethane using pre-treatment with 4% sodium hypochlorite

To remove the pigments, microalgal biomass was pre-treated with 10% sodium hypochlorite (v/v) that was diluted in water until a final concentration of 4% (v/v) was achieved. The microalgal biomass was mixed with 4% sodium hypochlorite and agitated for 20 min, and then centrifuged to separate the dead cells. The process was repeated and it was then rinsed twice with distilled water, followed by centrifugation and rinsing with acetone to facilitate drying. PHB was kept at 25 °C for 72 hours for evaporation of water[4].

Afterwards, extraction with trichloromethane at 65 °C for 4 hours was carried out, followed by filtration to remove ruptured cells. The trichloromethane was evaporated and the PHB precipitated with methanol. Methanol and PHB were centrifuged for separation and PHB was maintained at 25 °C for 72 hours for evaporation of water (Sample PHB3).

2.5 Extraction of PHB with trichloromethane using pre-treatment with 10% sodium hypochlorite

Sodium hypochlorite at 10% (v/v) and trichloromethane were added to the microalgal biomass, which was agitated for 150 min. After this, the mixture was separated into three phases using a funnel. The upper phase contained sodium hypochlorite, the central phase contained the ruptured cells and the lower phase contained trichloromethane and PHB[4]. The trichloromethane was evaporated and the PHB was precipitated with methanol. Methanol and PHB were centrifuged for separation. PHB was maintained at 25 °C for 72 hours for evaporation of water (Sample PHB4).

2.6 Preparation of solutions for electrospinning

Electrospinning tests were carried out with PHBs that were obtained from four extractions, using trichloromethane as a solvent. In the tests using PHB1, the samples were tested at concentrations of 20, 25, 30, 35, 40, 50 and 60% (w/v).

PHB2 was tested at concentrations of 25, 35 and 45% (w/v). This sample was also tested with the addition of *Spirulina* LEB 18 biomass. The concentrations of PHB2 and biomass were: 20% PHB2 and 5% biomass; 20% PHB2 and 10% biomass; 25% PHB2 and 5% biomass; 25% PHB2 and 10% biomass; 30% PHB2 and 10% biomass; 30% PHB2 and 20% biomass; 35% PHB2 and 10% biomass; 35% PHB2 and 15% biomass; 40% PHB2 and 10% biomass. All concentrations are in percentages (w/v).

In the process using PHB3, the solutions were prepared at concentrations of 15, 20, 25 and 30% (w/v). Solutions of PHB3 with the addition of *Spirulina* LEB 18 microalgal biomass were also tested. In these tests, 7% (w/v) PHB3 was used with 7% (w/v) biomass; 20% (w/v) PHB3 with 5% (w/v) biomass and 25% (w/v) PHB3 containing 5% (w/v) biomass.

In the tests with PHB4, electrospinning was carried out using concentrations of 15, 20 and 30% (w/v).

All the samples were homogenized in a magnetic agitator at 21 °C. The yield of PHB obtained through the different extraction methods was evaluated by analysis of variance (ANOVA) and Tukey's test to compare the means, at a 95% ($p \leq 0.05$) significance level.

2.7 Electrospinning process

In the electrospinning process, solutions were injected through a capillary with a diameter (D_c) of 0.45 to 1.10 mm. The distance between the end of the capillary and the collector (D_{cc}) was 150 mm. The electric potential (EP) that was applied ranged from 15.4 to 31.3 kV. The solution flow rate (FR) was between 0.7 and 5.8 µL min^{-1}, which was controlled by a pump connected to the pipette. All experiments were carried out at 21 °C.

2.8 Characterization of fibers

A scanning electron microscope (SEM) (Jeol JSM-7500F, Germany) was used to observe the morphology of the fibers. The mean fiber diameter was determined by measuring 30 different points on the SEM images. The apparent viscosity of all samples was determined using a viscometer (Haake PK100, Germany). The apparent viscosity and fiber diameter were assessed by analysis of variance (ANOVA) and Tukey's test for comparison of means at a significance level of 95% ($p \leq 0.05$).

The molar mass of polymers was determined by gel permeation chromatography (GPC) using a high resolution HPLC chromatograph (Knauer SL1000, Germany) equipped with a Polymer Standard Services column, and two detectors

(detector 1: Knauer V K2500 and detector 2: Knauer RI). Hexaisofluorpropanol (Aldrich, Germany) was used with solvent in the mobile phase at a flow rate of 0.5 mL min^{-1}. The system was maintained at 23 °C and 100 μL of solution was injected. Polystyrene (Aldrich, Germany) was used as the standard molar mass. The molar mass of the samples was determined with the aid of a standard curve.

The thermal degradation and degree of impurity of the polymers were measured using a thermogravimetric analyzer (TGA/SDTA851^e, Mettler, Germany). Approximately 10 mg of the sample was heated from 25 to 800 °C to 10 °C min^{-1} in a nitrogen atmosphere. The temperatures of onset and maximum degradation were determined by first derivative curves of TGA and the degree of impurities characterized by the amount of sample remaining at the end of the process.

Differential scanning calorimetry (DSC) was carried out to determine the degree of crystallinity (χ_C) of the polymers, by using crystallization enthalpy (ΔH_c) and fusion enthalpy using a calorimeter (DSC821^e, Germany) in a nitrogen atmosphere. Samples weighing approximately 10 mg were sealed in aluminum with heating and cooling between 25 and 200 °C to 10 °C min^{-1}. The degree of crystallinity was obtained by fusion enthalpy and crystallization enthalpy, considering that the fusion enthalpy for 100% crystalline PHB is 142 J g^{-1}[12].

3. Results and Discussion

3.1 Extraction yield

The PHB1 extraction method (4% sodium hypochlorite w/v) resulted in a greater ($p = 0.0002$) yield of polymer (30.1 ± 2%). There was no significant difference ($p > 0.85$) between the other extraction methods regarding the concentration of PHB obtained from the microalgal biomass, with values between 1.02% ± 0.20 and 1.71 ± 0.10% (Table 1).

According to Panda et al.,[13] the manipulation of culture conditions of the *Synechocystis* sp. PCC 6803 microalga increased the PHB content by six times. The largest accumulation of PHB in microalgae was 55%, which was observed in *Synechococcus* sp. MA19 when cultivated with a limited concentration of phosphate[14]. In this study, when *Spirulina* LEB 18 was cultivated, there was no imbalance, nor optimization of culture conditions to induce the production of polymers. However, the concentration obtained was between 30.1 ± 2% and 1.02 ± 0.20%, which shows that this microalga has a potential for PHB synthesis.

Extractions with sodium hypochlorite (samples PHB1, PHB3 and PHB4) produced yellow polymers, while the extraction with trichloromethane (PHB2), produced a green polymer. PHB granules are located near the thylakoid membrane, which is the site of photosynthesis energy generation. The proximity between the PHB granules and thylakoid membrane hinders the separation of PHB and photosynthetic pigments, especially chlorophyll *a*.[1] Thus, the extraction of PHB from microalgae requires certain adjustments to obtain polymer without pigments. However, the extraction process used in PHB2 can help to preserve the microalga's active components, which is extremely useful for the medical and food industries.

3.2 Degradation and melting properties

The PHB2 extraction had a maximum initial degradation temperature of 240.9 °C. The initial degradation temperatures of samples obtained from extractions PHB1, PHB3 and PHB4 were between 162.1 and 166.6 °C (Table 1). The samples had a maximum degradation temperature of between 453.7 and 484.7 °C. Sombatmankhong et al.[15] obtained a degradation temperature of 263.5 °C for PHB.

The PHB samples had a melting point between 171.9 and 126.3 °C, and the maximum melting point obtained was for the polymer extracted with trichloromethane. Sombatmankhong et al.[15] obtained a melting temperature of 172.6 °C for samples of commercial PHB. In nanofibers developed with PHB, Kim et al.[16] obtained a melting temperature of 165 °C.

The maximum degree of crystallinity was found in sample PHB2 (1.21%). However, this value is lower than that quoted in the literature for PHB, which is between 60 and 80% (Table 1). Therefore, the polymers that were obtained in this study should be considered to be amorphous. Crystalline polymers have better chemical resistance and are more brittle.

3.3 Molar mass

The maximum molar mass values obtained were $4.81,10^6$ Da (PHB2), $4.36,10^6$ Da (PHB1), followed by $3.85,10^6$ Da (PHB3) and $1.59,10^5$ Da (PHB4). During extraction of PHB from *Alcaligenes eutrophus*, with 30% hypochlorite and trichloromethane (1:1) for 90 min, Hahn et al.,[4] obtained a polymer with a molar mass of $3.0,10^5$ Da. In the extraction using trichloromethane, the molar mass of PHB was $5.3,10^5$ Da.[4] When Choi and Lee[3] extracted PHB from *Escherichia coli* with trichloromethane, NaOH and KOH they obtained a molar mass of $2.2,10^6$, $1.9,10^6$ and $2.0,10^6$ Da, respectively. The molar mass varied according to the microorganism used to extract the polymer, as well as with the conditions that the organism was exposed to and the extraction method used to recover PHB.

The degradation caused in the PHB2 molecules was insignificant, but the reagents used in the PHB1 extraction

Table 1. Method of extraction used to obtain PHB, yield (γ) obtained in the extractions and responses of initial temperature of degradation (TG_o), final temperature of degradation (TG_f), impurities (IP), melting temperature (T_m) degree of crystallinity (χ_c), mean molar mass (M_w) of polymers used in the development of fibers (mean ± standard deviation).

Extraction Condition	γ (%)	TG_o (°C)	TG_f (°C)	IP (%)	T_m °C)	χ_c (%)	M_w (Da)
PHB1	30.1 ± 2.0^b	162.1	464.0	31.3	126.4	0.31	$4.36,10^6$
PHB2	1.71 ± 0.1^a	240.9	453.7	34.4	171.9	1.21	$4.81,10^6$
PHB3	1.54 ± 0.5^a	166.6	484.7	26.8	127.5	0.17	$3.85,10^6$
PHB4	1.02 ± 0.2^a	163.5	461.4	30.5	126.3	0.23	$1.59,10^5$

Same letters in the same column indicate no significant differences between experiments ($p > 0.05$).

drastically decreased the molar mass[4]. These reagents caused further deterioration in cellular components due to the denaturation of nucleic acids and inactivation of enzymes. In this study, the method PHB4 resulted in PHB molecules with a lower molar mass.

3.4 Biomass impurities

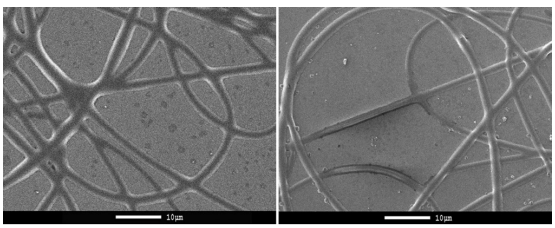
The TGA analysis showed that the PHB2 extraction had the maximum amount of non-PHB materials (34.4%), and PHB3 had the lowest concentration of these materials (26.8%). The samples obtained by extraction methods PHB1 and PHB3 had non-PHB concentrations of 31.3 and 30.5%, respectively.

Clinical studies suggest that compounds in *Spirulina* biomass have therapeutic functions, such as polysaccharides with an anti-inflammatory effect,[17] fatty acids with antibacterial and antifungal properties[18] and Calcium Spirulan, which has been reported to inhibit lung metastasis in humans, by preventing attachment and proliferation of tumor cells.[19] In an attempt to keep the active properties of microalgal biomass, purification processes were not carried out after the extractions. This led to materials other than PHB in the sample, which were identified as impurities.

3.5 Nanofibers obtained via electrospinning

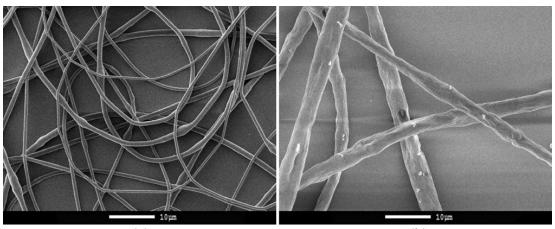
PHB2 and PHB3 presented uniform nanofibers, without droplets and stable electrospinning maintaining the continuity of the process (Figure 1 and Figure 2). Thus, these samples were selected for the development of nanofibers, incorporating the microalgal biomass of *Spirulina* LEB 18.

The samples of PHB1 and PHB4 not presented fibers. When the electrospinning test was carried out with lower concentration (20% PHB1 and 15% PHB4) there was no fiber formation, only drops (Table 2).



(a) (b)

Figure 1. Electron microscopy images (SEM) magnified 2,000 times, of nanofibers developed with solutions containing 35% PHB2 (a); and 25% PHB2 and 5% Spirulina LEB 18 microalgal biomass (b).



(a) (b)

Figure 2. Electron microscopy images (SEM) magnified by 2,000 times, of nanofibers developed with solutions containing 30% PHB3 (a); and 25% PHB3 and 5% Spirulina LEB 18 microalgal biomass (b).

In higher concentration (25 to 60% PHB1) solutions had fibers containing droplets and electrospinning was hindered by the obstruction of the capillary during the process. In the experiments containing 30% PHB4 was formed nanofibers with too much droplets, independent the condition tested in the electrospinning (Table 2).

The concentrations of the solutions of PHB2 and PHB3 that presented prospects of forming nanofibers were tested in different conditions of the electrospinning process (Table 3). The electrospinning conditions that had the best nanofiber characteristics during the process are presented in Table 3 asteriscked.

In selecting the best condition to form uniform nanofibers, the parameters were varied according to the need that was observed by the analyst. For example, in the sample containing 35% PHB2 was initially tested a random electric potential condition, feed flow rate, distance to the collector and the capillary and diameter of the capillary. Based on the results obtained in this experiment 1, it was observed that the increased electric potential could improve the characteristics of the nanofiber obtained. Thus, the electric potential was increased from 18.1 kV to 24.3 kV in the experiment 2. The changes made between the experiments are highlighted in Table 3 with the variation of colors. Therefore, several tests have been performed and the end of the experiments, microscopic observation of nanofibers showed that the best condition was 24.3 kV and 2 µl min^{-1}, distance from the capillary to the collector of 15 cm and a capillary diameter of 0.45 mm. All experiments were conducted in this way (Table 3).

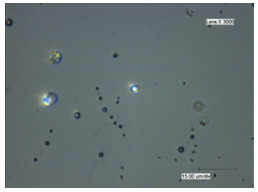
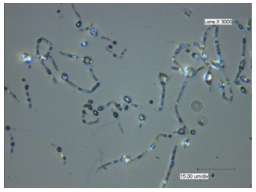
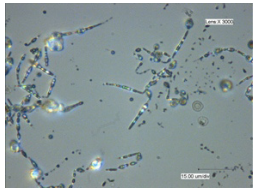
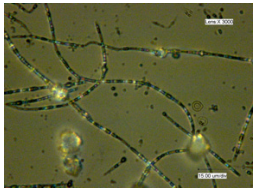
The smallest diameter of nanofibers was 826 ± 188 nm, obtained when a solution containing 30% PHB3 was used (Table 4). Suwantong et al.,[20] obtained nanofibers prepared with 14% PHB in trichloromethane with a diameter of 3,700 ± 1,700 nm. Solutions of 20% poly(3-hydroxybutyrate-co-valerate) in trichloromethane, had fibers with diameters between 1,000 and 4,000 nm[21].

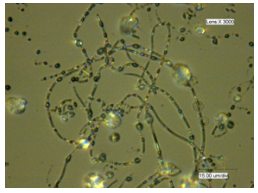
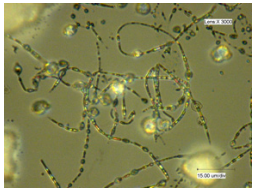
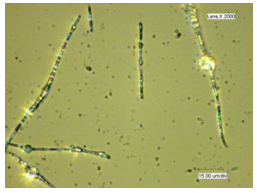
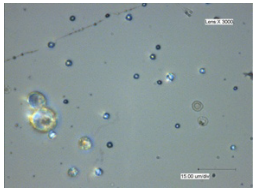
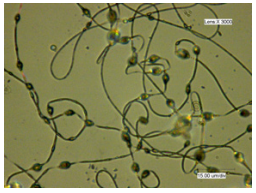
The nanofibers developed in this study had smaller diameters when compared to those of the authors previously mentioned. Smaller diameters cause the nanofibers to pack closer together with less distance between them. In the development of food packaging such a characteristic is favorable because it blocks oxygen, carbon dioxide and humidity. In tissue engineering, the small diameter increases the contact area, which stimulates adhesion, migration and proliferation of cells in nanofibers[21].

There was no significant difference regarding the diameter of the fibers for the other conditions tested. However, a faster flow rate of the solution and a larger capillary orifice produced an increase in the diameter of the fiber. In the sample containing 30% PHB3, which had a smaller nanofiber diameter (826 ± 188 nm), the flow rate was 2 µL min^{-1} and the capillary orifice was 0.45 mm. However, the maximum diameter (1,675 ± 194 nm) was obtained with 25% PHB3 and 5% microalgal biomass, using a 0.90 mm capillary diameter and a flow rate of 4.8 µL min^{-1} (Figure 1 and Figure 2).

The flow rate of the solution influences the speed of the jet and the rate of transference of nanofibers to the collector. Megelski et al.[22] observed that the diameter of nanofibers increases proportionally with the flow rate of the solution. A small inner diameter of the capillary reduces the diameter

Table 2. Conditions of the electrospinning process studied to solutions PHB1 and PHB4.

Experiment	20% PHB1	25% PHB1	30% PHB1			35% PHB1	40% PHB1
EP (kV)	18.4	18.4	24.4	24.4*	24.4	24.4	25.5
FR (μL min^{-1})	3.8	3.8	3.8	1.2	1	3.8	2.2
D_{cc} (cm)	15	15	15	15	15	15	15
D_e (mm)	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Electrospinning Results (3.000 X magnification)							

Experiment	45% PHB1	50% PHB1		60% PHB1	15% PHB4			30% PHB4		
EP (kV)	25.5	24.5	24.5*	24.5	18.6	24.5*	31.3	18.6	24.5	31.3
FR (μL min^{-1})	2.2	2.2	4.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
D_{cc} (cm)	15	15	15	15	15	15	15	15	15	15
D_e (mm)	0.45	0.45	0.90	0.60	0.45	0.45	0.45	0.45	0.45	0.45
Electrospinning Results (3.000 X magnification)										

The asterisk (*) indicates the best conditions with electrospinning result presented.

Table 3. The concentrations of the solutions PHB2 and PHB3 that presented uniform nanofibers (*) and all conditions of the electrospinning process studied.

35% PHB2					
Experiment	**1**	**2**	**3***	**4**	**5**
EP (kV)	18.1	24.3	24.3	31.3	31.3
FR (μL min^{-1})	2	2	2.5	2.5	3
D_{cc} (cm)	15	15	15	15	15
D_c (mm)	0.45	0.45	0.45	0.45	0.45
25% PHB2 + 5% Sp					
Experiment		6	7	8*	9
EP (kV)		18.1	24.1	31.2	31.2
FR (μL min^{-1})		2.5	2.5	2.5	2
D_{cc} (cm)		15	15	15	15
D_c (mm)		0.45	0.45	0.45	0.45
30% PHB3					
Experiment		10	11*	12	13
EP (kV)		18.1	25	25	31.2
FR (μL min^{-1})		2	2	1	1
D_{cc} (cm)		15	15	15	15
D_c (mm)		0.45	0.45	0.45	0.45
25% PHB2 + 5% Sp					
Experiment		14	15	16	17*	18
EP (kV)		18.1	24.3	24.3	24.3	31.1
FR (μL min^{-1})		2.5	2.5	4.8	4.8	4.8
D_{cc} (cm)		15	15	15	15	15
D_c (mm)		0.45	0.45	0.45	0.90	0.90

The different colors indicate where the change occurred between experiments.

Table 4. Characteristics and diameter of the nanofibers formed, and response of viscosity of solutions used for the development of fiber.

Concentration of solution (%)	Extraction conditions	Fiber characteristics	Diameter (nm)	Viscosity (Pa s)
30% PHB	PHB1	Broken fibers containing droplets	-	0.03 ± 0.00^a
35% PHB	PHB2	Uniform fibers	1,490±147.0^a	0.05 ± 0.02^a
25% PHB + 5% *Spirulina*	PHB2	Uniform Fibers	1,453±239.0^a	0.11 ± 0.05^a
30% PHB	PHB3	Uniform Fibers	826±188.0^b	0.11 ± 0.01^a
25% PHB + 5% *Spirulina*	PHB3	Uniform Fibers	1,675±194.0^a	0.24 ± 0.04^b
30% PHB	PHB4	Fibers with droplets	-	0.01 ± 0.00^a

Same letters in the same column indicate experiments between which there was no significant difference ($p > 0.05$) (mean ± standard deviation).

of the nanofibers. With a capillary diameter of 0.84 mm and PEO concentration of 7%, Son et al.,[23] obtained nanofibers with diameters ranging from 360 to 1,960 nm.

There was no significant difference ($p > 0.15$) in viscosity between the solutions that provided the best conditions for electrospinning (Table 4), except for the sample containing 25% PHB3 and 5% microalgal biomass, which had nanofibers with a maximum diameter. If the viscosity increases, the diameter of the nanofibers also increases due to the solution's greater resistance to stretching.[8]

The viscosities of the solutions ranged from 0.010 ± 0.002 to 0.240 ± 0.040 Pa s, with solution with concentrations around 30% (Table 4). Sombatmankhong et al.,[15] obtained viscosities from 0.43 to 2.30 Pa s, for PHB samples with concentrations between 10 and 16% (w/v).

The viscosity can be increased by increasing the concentration of the polymer. This results in polymer chain links within the solution, which makes the jet continuous during electrospinning[8]. In this study, the extracted polymers had lower viscosity values when compared with polymers quoted in the literature, but this was offset by the increase of the concentration of the solution, which enabled the development of nanofibers using the electrospinning process.

4. Conclusions

The *Spirulina* LEB 18 microalga had a maximum concentration in PHB1 (30.1 ± 2%). The smallest diameter of nanofibers developed was 826 ± 188.0 nm, obtained in PHB3 with 30% solution.

The PHB2 extraction method produced a polymer with a green color and with maximum content of non-PHB materials (34.4% impurities). This is a desirable outcome for the application of nanofibers in the medical and food fields, because active components in the microalga are preserved and these may assist in cell growth and the development of edible packaging.

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