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Obtainment of Polyhydroxyalkanoates (PHAs) from Microalgae Supplemented with Agro-Industry Residue Corn Steep Liquor

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Abstract

In this study, it was possible to evaluate the production of polyhydroxyalkanoates (PHAs) from the microalgae *Chlorella vulgaris* and *Tetradesmus obliquus*, using 1% and 0.25% (v/v) of agro-indusytial Corn Steep Liquor residue, a product from corn processing. The biomass was subjected to mechanical extraction using 4% sodium hypochlorite + chloroform, and the extract was further characterized using Fourier Transform Infrared Spectroscopy (FTIR) analysis. It was possible to observe stretching common to PHAs described in the literature, such as the strong vibration of the carnonyl ester group (C=O) at 1735 cm^{-1} and 1720 cm^{-1}, and the presence of methyl (CH3) and methylene (CH2) groups, for *C. Vulgaris* and *T. obliquus*, respectively. Therefore, these indications suggest that both microalgae are potential producers of polyhydroxyalkanoate biopolymers, and have possible applications in the production of bioplastics. This is the first report on the production of polyhydroxyalkanoates from these microalgae, especially the isolated strain, *Tetradesmus obliquus*.

Keyword

Bioplastic, Polyester, Polyhydroxyalkanoate, Corn Steep Liquor, *Chlorella vulgaris, Tetradesmus obliquus*

Introduction

Polyhydroxyalkanoates (PHAs) are biopolymers produced by a range of microorganisms, including microalgae, and will be great replacements for conventional petroleum-derived plastics as they have properties similar to synthetic polymers and are biodegradable [1], capable of forming plastic membranes. Bioplastics obtained from renewable biomass have already been produced from first-generation raw materials such as sugar beet, cornor second-generation raw materials such as lignocellulose materials. Currently, more attention is being paid to production from microorganisms, theso-called third generation, which do not compete with human, animal, fresh water or arable land [2,3]. The microbial production of PHAs has been reported for decades, however it has been gaining a lot of attention in recent years, due to the wide variety of applications of these biopolymers in several sectors. However, there is a concern with its production, due to the high costs of organic substrates used for PHA production by heterotrophic bacteria. Thus, the use of photosynthetic microorganisms such as microalgae and cyanobacteria can become quite advantageous, since these microorganisms do not require costly nutritional sources and have minimal nutritional requirements such as in organic sources (CO_2, N, P) and light [4].

Materials and Methods

Microalgae and growing conditions

Two microalgae were used in the experiment and both were cultivated in standard Bold's Basal medium [5]. *Chlorella vulgaris* UTEX 1803 was cultivated at 27 ± 1 °C, constant lighting (3800 lux), constant aeration and supplemented with 1% corn steep liquor (v/v) [6]. *Tetradesmus obliquus* was grown at 27 ± 2 °C, constant illumination (3000 lux), constant aeration and supplemented with 0.25% corn steep liquor (v/v) [7]. *C. Vulgaris* is a commercial strain (University of Texas, Austin) and *T.obliquus* was isolated from Açude of Apipucos (Recife, Pernambuco, Brazil, coordinates 8°1’13.08” S; 34°55’ 56.51” W).

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Treatment of corn steep liquor

Corn Steep Liquor was kindly provided by the company Ingredion (Cabo de Santo Agostinho, Pernambuco, Brazil), was treated in accordance with Liggett and Koffler [8], with slight modifications. Initially, Corn Steep Liquor was centrifuged at 8000 rpm for 10 minutes to remove solid particles and then concentrated NaOH was added to adjust pH 8 and autoclaved at 121 °C for 20 minutes. The agroindustrial residue was then centrifuged again and the supernatant was used on the cultures.

Biopolymer extraction

1.0 g of lyophilized biomass of *C. Vulgaris* (CHLOB) and

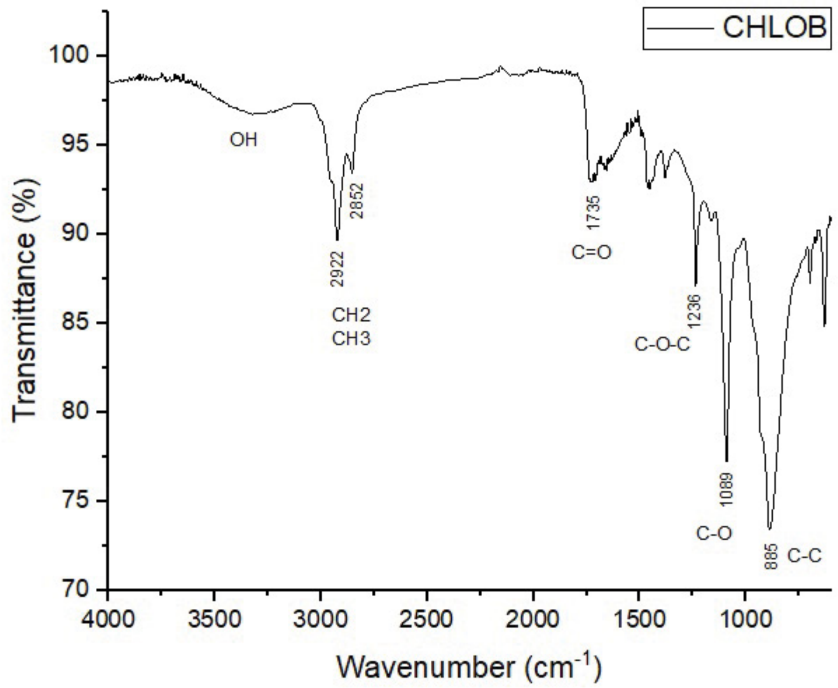

Figure 1: FTIR/ATR spectrum of *C. Vulgaris* extractcultivated in 1% corn steep liquor. The spectrum was recorded ranging from 4000-600 cm^{-1}.

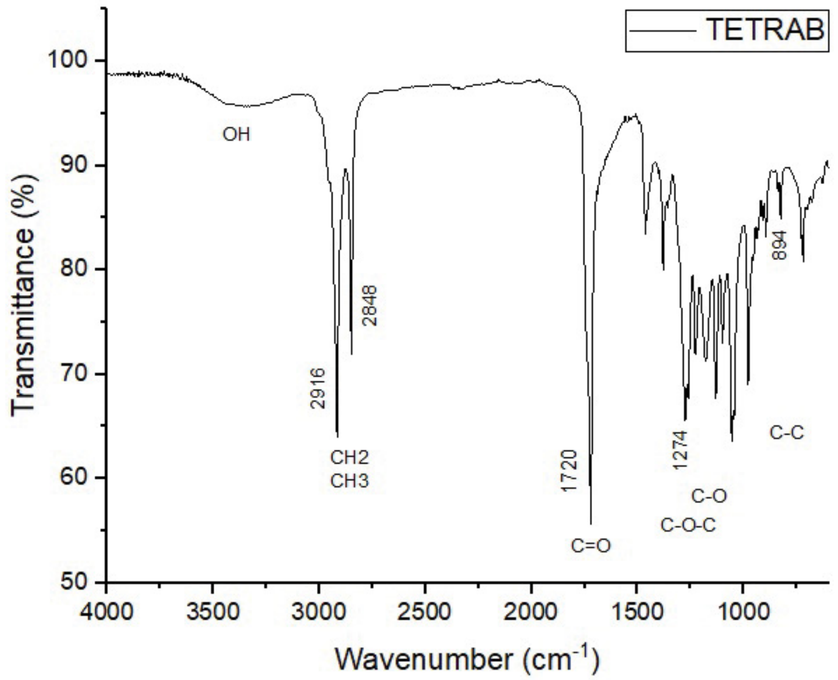

Figure 2: FTIR/ATR spectrum of *T. Obliquus* extractcultivated in 0.25% corn steep liquor. The spectrum was recorded ranging from 4000-600 cm^{-1}.

T. Obliquuos (TETRAB) were subjected to motor agitation with a solution of hypochlorite (4%) + chloroform (v/v), then the samples were centrifuged (3000 rpm, 10 minutes, temperature environment) and the organic phase was collected, evaporated, degreased with hexane and subjected to spectroscopic analysis.

Fourier Trasnform Infrared Spectroscopy (FTIR)

Samples of PHAs were qualitatively analyzed with Fourier transform infrared spectroscopy (FTIR, Shimadzu, model IR-Tracer 100) between 4000 and 600 cm^{-1} using ATR accessory with a zinc selenide crystal.

Results and Discussion

Fourier Transform Infrared Spectroscopy (FTIR) analysis of PHA samples

Figure 1 and Figure 2 show the spectra of the obtained PHA samples. In the figures it is possible to observe how transmittance bands at 1735 and 1720 cm^{-1} are attributed to strong vibration of the carbonyl ester group (C=O), strongly indicative of medium chain length PHAs (mcl-PHAs), corroborating with Giaquinto, et al. [9] who say that PHBs have bands corresponding to the carbonyl ester group ranging between 1720-1650 cm^{-1}. Other characteristic signs of PHAs are also observed in the spectra, such as the presence of elongations of the –OH group within the carboxyl group, ranging between 3400-3200 cm^{-1}, methyl groups (CH_3) and methylene group (CH_2), with spectra ranging between 2961-2854 cm^{-1}, flash bands between 1466-1000 cm^{-1} are attributed to strong vibration of the C-O group, and other characteristic bands present that are attributed to the C-C group [10,11]. All these indicatives presented as microalgae studied in this work have the ability to synthesize biopolymers capable of forming plastic membranes. Roja, et al. [11] analyzing the elongations obtained in the FTIR of 4 types of algae (Chlorella sp., *Oscillatoria salina*, *Leptolyngbya valderiana* and *Synechococcus elongatus*) obtained wave number of the C=O group varying between 1625-1644 cm^{-1}. Costa, et al. [12] evaluating different methods of extraction of the biopolymer obtained from *Spirulina* sp. obtained a strong vibration of the carbonyl ester group (C=O) ranging from 1735-1745 cm^{-1}, corroborating the present study.

Conclusion

The results show that the use of maize for the production of polyhydroxyaalkanoates from the microalgae *Chlorella vulgaris* and *Tetradesmus obliquus* is possible. In the spectroscopic analysis (FTIR) it was possible to observe the main characteristic elongation of the PHAs, with a strong vibration of the est carbonyl group (C=O), in addition to the methyl (CH_3) and methylene (CH_2) groups. Therefore, microalgae have a strong industrial potential in the production of plastic biofilms.

Acknowledgements

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Conflicts of Interest

None.

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