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Evaluation of the Types of Starch for Preparation of LDPE/Starch Blends

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ABSTRACT

This study evaluated in relation the growth, and the amylolytic activity of mixed and isolated cultures of Phanerochaete chrysosporium *and* Talaromyces wortmanni *on different types of starch. The thermal and mechanical properties in polyethylene/starch blends (proportion: 80/20 (w/w) before and after inoculation of the mixed cultures were evaluated. The regular starch Amidex 3 and the modified starch Fox5901 stood out in relation to the cellular growth and production of the amylase enzyme. In spite of the short time that the blends were exposed to the fungi, the microorganisms promoted physical and chemical changes in the structure of the blend, modifying its thermal and mechanical properties. The alteration of the degree of crystallinity and mechanical properties of the blends could be indications of the modification caused by the biodegradation process.*

Key words: *Amylolytic enzymes; polyethylene; starch; blends; Talaromyces wortmanii; Phanerochaete crysosporium*

INTRODUCTION

The scientific knowledge and the technological advances in the field of polymers have enabled the development of several applications of these materials. However, the accelerated consumption of polymers, mainly in the applications of disposables, like for example packaging, has roused strong concerns due to the generation of residues from the polymeric materials.

Biodegradable polymers are recently developed materials in the field of polymers. Their main characteristic is that they are biodegradable through the action of the microorganism in appropriate environmental conditions. When in contact with the biodegradable polymer, the microorganisms produce enzymes that break down the material in progressively smaller segments; that is to say, they reduce its average molecular mass, favoring its degradation in the environment (Dupret, et al., 1999; Bonhomme et al., 2003; Nakamura et al., 2005). Studies are being conducted to prepare new thermoplastic materials, composed of blends of synthetic polymer with natural polymer, that are degraded more easily when discarded in the environment (Chandra and Rustgi, 1996; Cha and Pitt, 1989; Pedroso and Rosa, 2004).

There is great interest in incorporating biodegradable materials such as starch into conventional plastics such as polyethylene (Kim, 2003; Rodriguez-Gonzalez et al., 2003; Matzinos

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et al., 2002; Baldev et al., 2001). Starch is a polysaccharide that is gaining great importance in the field of new materials and the environment. Besides improving the biodegradable capacity of the microorganisms, the type of starch used in the making of polymeric blends can interfere directly in the properties of the polymer (Thakore et al., 2001).

The polymeric system composed of a mixture of the conventional plastic with the biodegradable polymer is believed to produce a type of plastic material with different mechanical properties, presenting resistance to heat, light and humidity. When this material is discarded into the environment, it can be degraded by microorganism whose natural habitat is the same soil (Ratto et al., 1999; Lima, 2005; Nakamura et al., 2005).

In this paper we present a study of the different types of starch in relation to the growth and the amylolytic activity of mixed and isolated cultures of the fungi *Phanerochaete chrysosporium* and *Talaromyces wortmannii*. The thermal and mechanical properties of the polymeric blends of polyethylene/amphoteric starch through the action of mixed cultures of these fungi were also evaluated. The aim was to compare the biodegradation process performance for both systems.

MATERIAL AND METHODS

Polymer

A polyethylene of low density (LDPE) was the polymer used; it was supplied by BRASKEM. The different types of starch were supplied by Corn Products of Brazil (Table 1). Few works used modified starches to obtain films from polyethylene blends with starch (Shah, Bandopadhyay and Bellare, 1995; Zuchowska et al. 1998; Thakore et al. 2001; Rodríguez-Gonzalez et al. 2003). The amphoteric starch with the N^+ and P^- ions and the starch Fox 5814 with cation N^+ were chosen.

Table 1- Different types, sources and characteristics of the starch.

Product	Type of Starch	Source of Starch	Characteristics
Amidex® 3	Regular	Hybrid corn	High Mw and Viscosity, retrogrades
Amidex® 4	Regular	Corn waxy	High Mw and Viscosity, no-retrogrades
Amisol®	Dextrin	Hybrid corn	Low Mw and Viscosity
Fox® 5814	Cationic	Hybrid corn	High substitution degree
Fox® 5901	Amphoteric	Hybrid corn	Low substitution degree

Obs: Hybrid corn = 27% amylose and 73% amylopectin
Waxy Corn = 100% amylopectin
Mw= Molecular weight

Microorganism

A strain of *Phanerochaete chrysosporium* (Ph) from the culture collection at the Federal University of Rio de Janeiro- Brazil and a wild strain of *Talaromyces wortmannii* (BM-10) isolated from the soil of the Muribeca Landfill (Jaboatão dos Guararapes - PE - Brazil) were used.

Maintenance of the fungus cultures

The strains of fungi were maintained in a Sabouraud-agar medium containing (g/L): peptone (10), glucose (40), NaCl (7.5), meat extract (3.5), agar (12) and distilled water (1L). The pH was adjusted to 4.5 and the sterilization was accomplished at 110°C for 20 minutes. The strains were stored at 5°C.

Biomass

The mixed and isolated fungus cultures in different types of starches were cultivated in Erlenmeyer flasks containing a Sabouraud-agar medium of 25/75% w/w starch/glucose and 100% starch. The initial spore concentration was 10^6 spores/mL. The growth was obtained through agitation at 200rpm at room temperature during 14 days, then the content of each flask was filtered through J.PROLAB No 0,007 filter paper. Harvested biomass was washed twice with 200 mL of distilled water and dried at 105 $\pm$ 1^{o}C. This experiment was carried out in triplicate.

Amylolytic activity

The test for detection of the amylases formation was accomplished by cultivating the fungi in petri dishes containing a Sabouraud-agar medium of 25 and 100% starch, respectively. The mixtures were prepared for the substitution of the glucose in weight for the respective ones percentile of starch in the form of fine powder. Each dish was inoculated with a platinum needle and incubated at 28°C for 7 days. Amylolytic activity was validated, in triplicate for each fungus, through the addition of metallic iodine producing a blue coloration in the presence of starch. For quantitative evaluation of the amylase enzyme the respective diameters of de cycle and the colony were measured (Lima, 1999; Rodrigues and Garcia, 1993). The areas of the degradation circle were calculated applying the formulas:

$$S = (d_c/2)^2 - (d_{co}/2)^2$$

Where: S = area of the circle or total activity; d_c = Diameter of the circle; d_{co} = Diameter of the colony.

Processing of the film

In this study LDPE/starch blends were utilized, in the proportion of 80/20 (w/w), mixed by fusion in the mixture chamber of a HAAKE 90 rheometer under the following conditions: control temperature: 140°C; maximum torque: 50 Nm; rotor velocity: 50 rpm; total time of mixing: 10 min. The LDPE/starch blend resins, which were processed in the rheometer, were triturated and compressed in a hydraulic press for the obtaining of films perfectly homogeneous with thickness of the 17.5$\pm$2.5mm.

Biodegradation activity test in polymeric blends

The mixed culture was tested with regard to their ability to degrade polymeric films according to the methodology developed by Lee et al. (1991) (Rosa et al., 2001; Lima, 2005). The modification in the polymeric structure was investigated through the thermal properties of previously degradation the films by Differential Scanning Calorimetry (DSC) and mechanical tests.

Differential Scanning Calorimetry (DSC)

A Thermal properties study was conducted using a Shimadzu thermal analyzer with a DSC TA-50 module in a nitrogen atmosphere. Samples of 5-10 mg were sealed in aluminium dishes and subjected to heating-cooling cycles. The sample was heated up at a rate of 10^{o}C /min.

Mechanical test

The mechanical tests were conducted in an EMIC DL 500 MF universal testing machine, observing the ISO 37 standard. On the basis of this analysis, the tensile strength and elongation at break percentage were determined.

All results obtained were appraised through statistical analyses, using the program STATISTIC 22 (1998).

RESULTS AND DISCUSSION

The samples were analyzed in triplicate, and Student's *t* test was applied to evaluate the statistically significant differences in their properties (Miller and Miller, 1984).

The *Phanerochaete chrysosporium* (Ph) was chosen since this fungus has been used in works concerning with the biodegradation of polymeric blends (Aitken and Irvine, 1989; Eriksson et al., 1990; Orhan and Buyukgungor, 2000). The strain *Talaromyces wortmannii* (BM-10) was chosen because it was not a pathogenic fungus besides it is the second species more common of the existent in soil, having been isolated in several continents (Domsch and Gams, 1980). Whereas the other isolated strains from the Landfill soil were.

Selection of the starch

Fig. 1 presents the biomass of the isolated and mixed cultures of *chrysosporium* and *wortmannii*, in the different types of starch, in a culture medium containg 25/75% w/w starch/glucose. *chrysosporium* presented favorable growth in the medium containg starch of the amidex 3 and Fox5901 types (Fig. a). Apparently the structural characteristics of those starches favored the growth of fungus. Amidex 3 is a regular starch extracted from hybrid corn, composed of 25% amylose and 75% amylopectin. In this type of starch, there is an amorphous and crystalline area, due to the presence of amylopectin and amylose, respectively. The enzymatic attack possibly favored the liberation of the glucose initially in the disordered area in the structure of the starch, which was consumed as a source of complementary carbon.

The Fox5901 starch is a modified starch that contains in its structure a cation (N^+) and an anion (P^-). *chrysosporium* presented favorable growth in this type of starch (Fig. 1a). It was believed that besides carbon source, the other nutrients such as phosphorus and nitrogen mighty have contributed to the consumption of this type of starch.

The behavior of *wortmannii* in the medium containing 25% starch demonstrated that the starches Amidex 3 and Fox5901 were responsible for the most favorable biomass production when compared with the other types of starch (Fig. 1b). The mixed culture presented better growth in the culture medium containing 25% regular starches (Amidex 3 and Amidex 4). However the biomass production in the modified starches, the cationic starch and the amphoteric starch, resulted in the same statistic results (Fig. 1c).

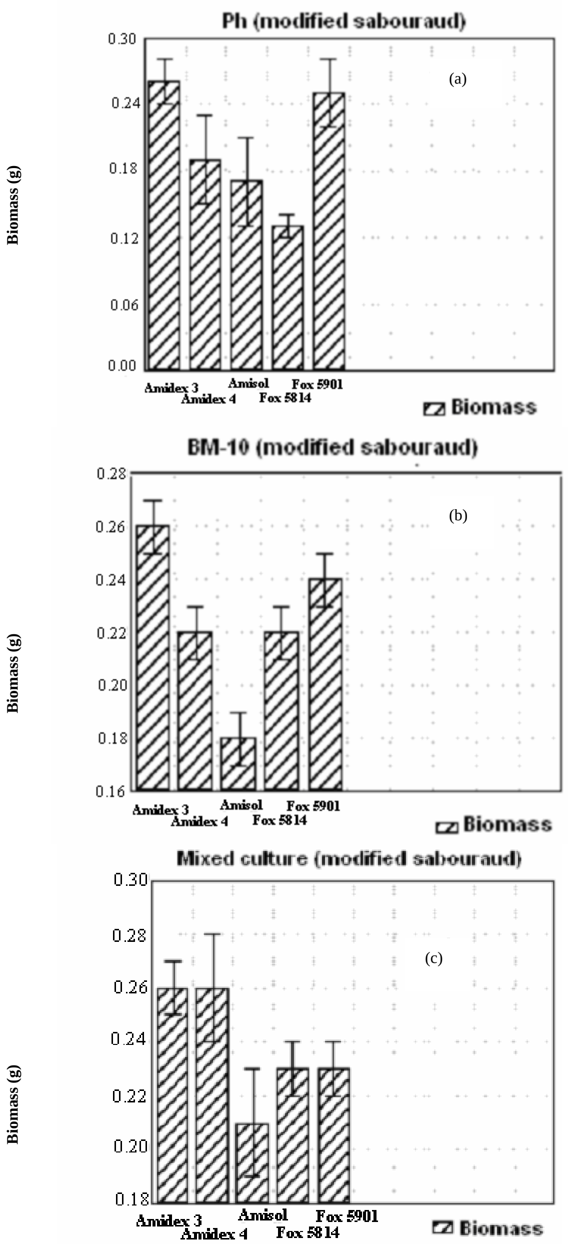
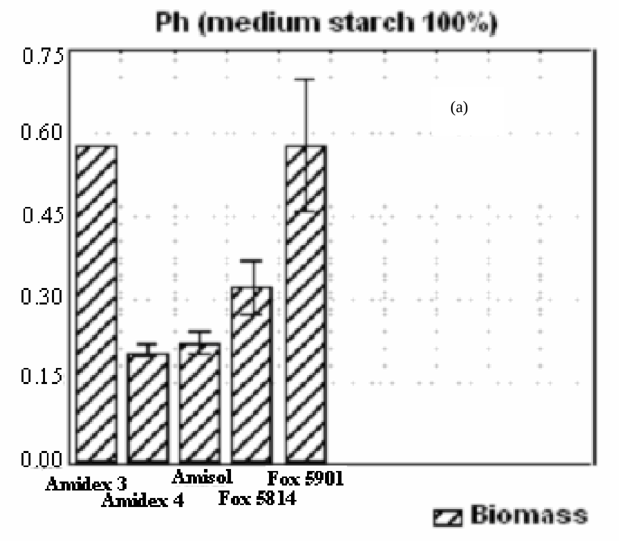
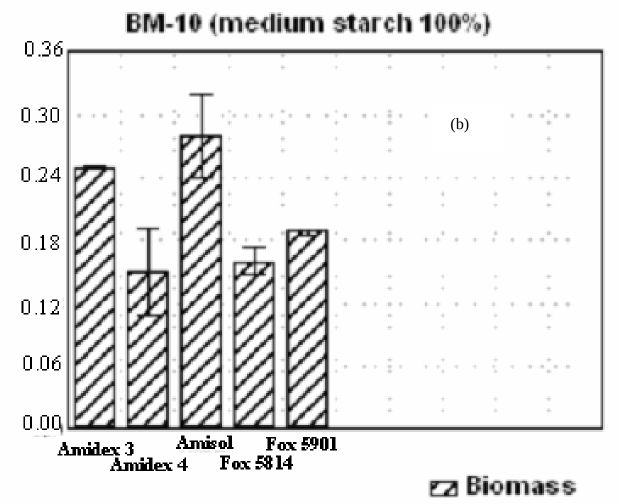


Figure 1- Biomass of the isolated cultures of (a) *Phanerochaete chrysosporium*, (b) *Talaromyces wortmannii* and (c) mixed culture, respectively, in the different types of starch/glucose.

Fig. 2 represents the biomass of the isolated and mixed cultures of *chrysosporium* and *wortmannii*, in a medium of 100% starch. Results showed that the isolated and mixed cultures of the fungi produced more biomass in the medium of 100% starch than in the medium of 75% glucose and 25% starch (Fig. 1). *chrysosporium* presented the same biomass production (0.59g) in cultivation media Amidex 3 and Fox5901 (Fig. 2a). *wortmannii* produced the largest quantity of biomass (0.28g) in the medium containing the Amisol starch (Fig. 2b). This favorable cellular growth could have occurred because it was a starch of low molecular weight. However, the growth of the mixed culture (Fig. 2c) was very satisfactory in the studied types of starch, distinctly indicating as the best source of carbon the regular starches (Amidex 3 and Amidex 4).





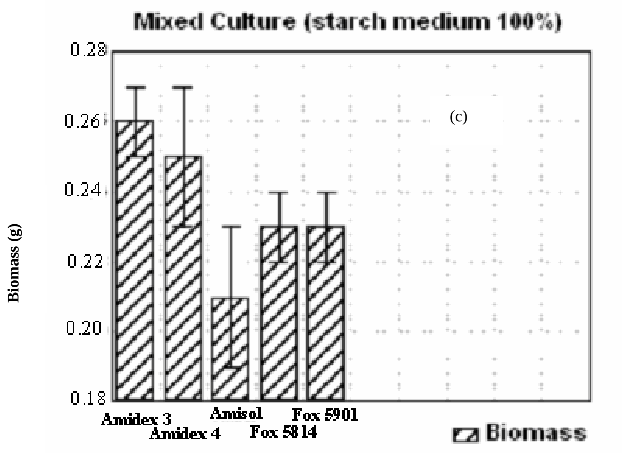


Figure 2- Biomass of the cultures of (a) *Phanerochaete chrysosporium*, (b) *Talaromyces wortmannii* and (c) mixed culture, respectively, in the different types of starch.

One of the important factors in justifying the cellular growth of the fungi in different media of starch was the evaluation of the production of the amylase enzyme. Amylases are enzymes that hydrolyze starch molecules resulting in a variety of products of low molecular weight, composed of units of glucose, which are consumed as a carbon source by the fungi (Dey et. al. 1991; Roldán-Carrillo et al. 2003). Microorganisms produce two categories of amylase: endoamylases and exoamilases. Endoamylases catalyze hydrolysis in the inner of the starch molecule randomly. This causes the formation of linear and branched oligosaccharides of various chain lengths. Exoamylases hydrolyze from the non-reducting end, successively resulting in short end products (Gupta, et al., 2003). Fig. 4 shows the amylase production by the *wortmannii* in different types of starch over a period of 7 days. Fig. 3a represents the amylase production in the medium containing 25% starch and 75% glucose. The production was more pronounced in the medium containing the regular starch Amidex 3 (3.3 cm^2). According to Fig. 3b, the enzyme production in the medium containing 100% starch also induced a higher production of this enzyme brought about by the fungus. The regular starch Amidex 3 (3.3 cm^2) and the modified starch Fox5901(3.0 cm^2) were distinctively indicated as favorable.

In all the types and compositions of starch, *chrysosporium* presented a fast cellular growth over the whole area of the dish, complicating the detection of the amylase production. The results obtained on the cellular growth and amylase production by *chrysosporium* and *wortmannii* in the medium containing 25 and 100% starch were satisfactory. Therefore, they indicated that the types of starch studied were able to compose biodegradable polymeric blends.

Evaluation of the biodegradation of the blends

For the evaluation of the biodegradation of the polyethylene/starch blend by the mixed culture of the fungi, the Fox5901 starch was used. To evaluate the compatibility of the polyethylene and starch mixture, thermal analyses of the starch, polyethylene and polyethylene/starch were carried out. Fig. 4 presents the thermal analysis of the LDPE, starch and polyethylene/starch blends.

The LDPE (Fig. 4a) showed a glass transition temperature of 83°C and a melting temperature of 115°C, whereas the amphoteric starch Fox5901 presented a melting temperature of 64°C (Fig. 4b). The mixture of LDPE with starch (Fig. 4c), in the proportion of 80/20 w/w, resulted in a compatible blend, with only one melting temperature (107°C), thus characterizing a new type of polymeric material, with a predominantly crystalline area.

The results of DSC on the polyethylene/starch blends submitted to biodegradation test with the mixed culture of the *chrysosporium* and *wortmannii* for 30 days presented a small variation in the melting temperature, as well as a decrease in the fusion temperature, which indicated an alteration of the degree of crystallinity of the polyethylene/starch mixture (Table 2), deriving from the degradation of the blends by the fungi when compared to the blends without the presence of the fungi (standard).

Table 3 shows the results of the mechanical tests of the polyethylene/starch blends before and previously submitted to biodegradation test. The blends that were inoculated with the fungi (A_1), presented a 32% decrease in the tensile strength, whereas the elongation at break presented a 5% increase when compared to the blends that were not inoculated with the microorganisms (B). There was no significant difference in the mechanical properties of the blends without inoculation (A_0) of the fungi with the standard (B).

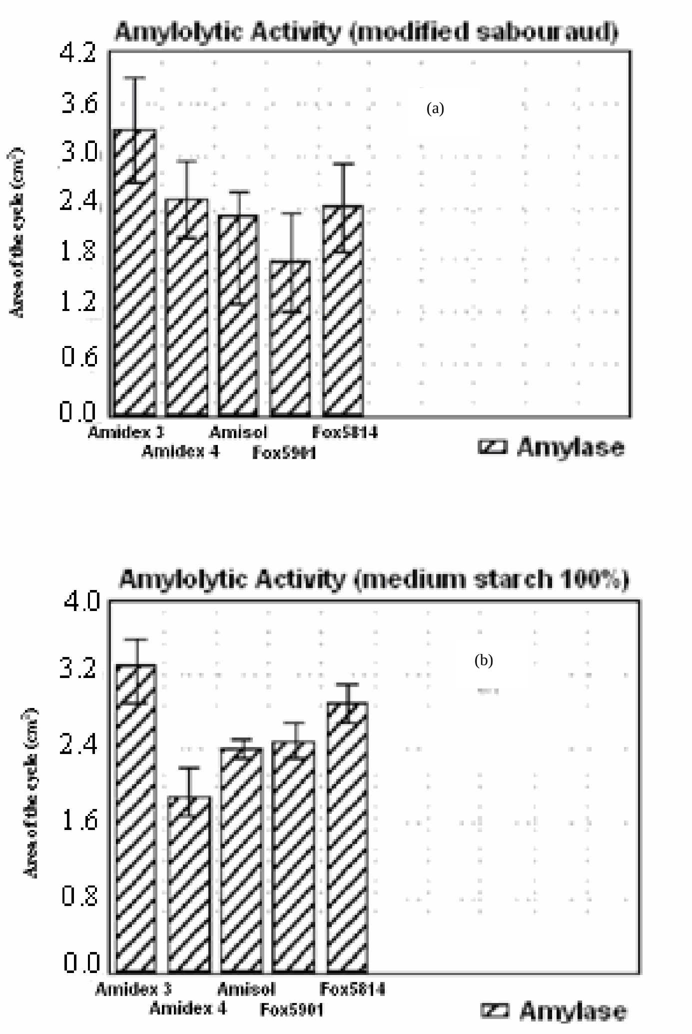


Figure 3 - Amilolytic activity of the *Talaromyces wortmannii* in the medium modified Sabouraud (10g starch/10g glicose).

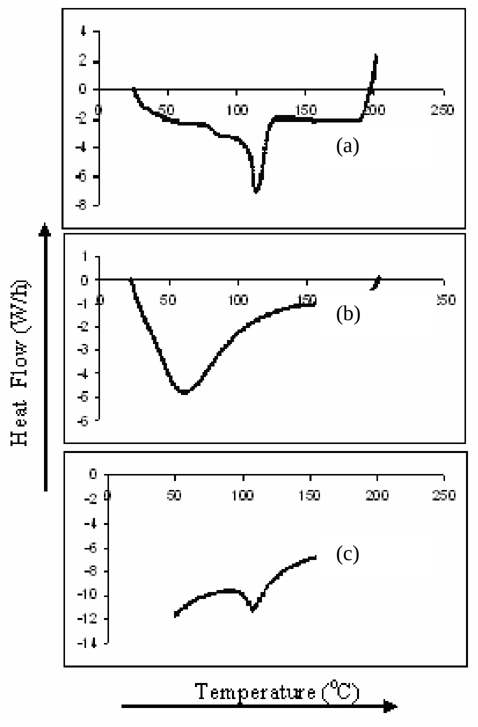


Figure 4 - Thermal analysis of the a) LDPE, b) starch and c) LDPE/starch blend.

Table 2- Results of the thermal analysis of LDPE, Starch, and polyethylene/Starch blends before and previously submitted to biodegradation test.

Sample	Incubation period (days)	T_m (°C)	ΔH_m (W/h)
Ao	0	107,5	11,2
B	30	107,4	18,9
A_1	30	109,8	9,17

A_0 = polyethylene/ starch blend without inoculation.
A_1 = polyethylene/ starch blend after inoculation of the fungi, period 30 days.
B = standard (polyethylene/ starch blend in the same conditions of A1 without the presence of the fungi).

Table 3- Results of the mechanical properties of the polyethylene/starch blends before and after the biodegradation.

Sample	Incubation period (days)	%El	σ (MPa)
A_0	0	30.65±0,26	5.69±0,54
B	30	31.06±0,56	4.70±0.56
A_1	30	32.48±0,35	3.22±0.33

A_0 = polyethylene/ starch blend without inoculation.
A_1 = polyethylene/ starch blend after inoculation of the fungi, period 30 days.
B = standard (polyethylene/ starch blend in the same conditions of A1 without the presence of the fungi).
%El = percentage elongation at break.
σ = Tensile strength

It could be believed that with the increased time of exposure of the blends to the fungi, the modifications of the mechanical and thermal properties should be more accentuated since the microorganisms would continue to act on the biodegradation process. This hypothesis was based on the Sturm method results that followed the production of CO_2 (Rosa et al., 2001; Lima, 2005).

CONCLUSION

The cellular growth of the isolated and mixed cultures of the *chrysosporium* and *wortmannii*, in the different types of starch in the culture medium containing 25 and 100% starch was studied. The regular starch Amidex 3 and the modified starch Fox5901 presented better performances. *wortmannii* proved to be a good producer of the amylase enzyme in different types of starch and in the culture medium containing 25 and 100% starch. The regular starch, Amidex 3 and the modified starch Fox5901 presented better results.

In spite of the short time that the blends were exposed to the fungi, it was concluded that the microorganisms promoted physical and chemical changes in the structure of the blend, modifying its thermal and mechanical properties. The alteration of the degree of crystallinity and the decrease in the tensile strength as well as the increase in the percentage of elongation at break, indicated the modification caused by the biodegradation process.

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RESUMO

Nesse trabalho foi realizado um estudo sobre diferentes tipos de amido quanto ao crescimento, e a atividade amilolítica de culturas mistas e isoladas dos fungos *Phanerochaete chrysosporium* e *Talaromyces wortmannii*. Avaliaram-se também as propriedades térmicas e mecânicas das blendas de polietileno/amido anfótero (na proporção 80/20 (m/m)) antes e apos a inoculação das culturas mistas desses fungos.O amido regular Amidex 3 e o amido modificado Fox5901 foram os que se destacaram quanto ao crescimento celular e produção da enzima amilase. Apesar do pouco tempo de exposição dos filmes com os fungos, pode-se concluir que os microrganismos promovem mudanças físicas e químicas na estrutura da blenda, modificando suas propriedades térmicas e mecânicas. A alteração do grau de cristalinidade e das propriedades mecânicas das blendas podem ser indícios da modificação provocada pelo processo de biodegradação.

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