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Effects of gamma radiation on enzymatic production of lignolytic complex by filamentous fungi

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This work aimed to study the effect of gamma radiation on the production of enzymes by filamentous fungi present in the seawater used for thermoelectric Termope S / A, in the vicinity of Port of Suape, Pernambuco. The isolated microorganisms were screened for their ability to produce enzymes. Subsequently, the fungi were irradiated at 3 doses (500, 1000 and 1500 Gy), using 4 inoculation techniques: lyophilisation, scraping of the spores, glass beads and agarose block. A fermentation assay for enzyme production was made in simple batch using a bioreactor New Brunswick, model Bioflo 110. The best enzyme produced was lignin peroxidase (676 U/L) by *Aspergillus awamori* in 500 Gy dose. Manganese peroxidase enzyme (1720 U / L), produced by three species of fungi (*Penicillium* sp.), was the highest in 500 Gy dose. *Aspergillus terreus* produced laccase enzyme (980 U/L) better in 500 Gy dose. In the assay of the fermentation bioreactor, the best results were found in BH-amid corn steep liquor, where 650 U/L of laccase was produced at the baseline and also in the medium containing corn steep liquor, where 620 U/L laccase was produced during 72 h. This is better than Sabouraud. The use of gamma radiation increased the production of enzymes by filamentous fungi compared to tests without radiation. Statistical analysis shows there are no significant differences between the inoculation techniques, and the best dose of radiation tested was 500 Gy. In trial bioreactor containing Bushnell Haas + corn steep, *Penicillium* sp. produced larger amounts of phenoloxidases enzymes compared to Sabouraud medium. Irradiation in a dose of 500 Gy stimulates *A. awamori*, *A. terreus* and *Penicillium* sp. to produce phenoloxidases enzymes.

Key words: Aspergillus, penicillium, corn steep, radiation.

INTRODUCTION

Marine biotechnology is considered as an area of great interest because of its high potential and contribution to the construction of an eco-sustainable society. Aquatic environment is not yet fully explored and its resources can play an important role in many industrial activities. Different research priorities can be identified in the field of marine biotechnology to show the vision of evolution and perspectives for the coming years (Melamed et al., 2002;

Adams, 2006; AS, 2009). The fungi present in marine ecosystems are associated with different bodies. However, these microbial groups are still not fully relatively studied in terms of their ecological functions and evolutionary origin as sources of useful metabolites for medicine, agriculture or industry (Osterhage et al., 2002; Klemke et al., 2004). Those considered as marine fungi are of biotechnological interest, since they produce metabolites such as enzymes. Microorganisms are a major producing sources of enzymes used in industrial society. Attractive and cost effective production of these metabolites is considered source and may be grown in large quantities and in a relatively short time (Zimmer et al., 2009). Enzymes are biocatalysts used in industry, and can be used in molecular biology, biomedical applications (Demain and Adrio, 2008), for the development of analytical methods for product manufacturing and technological treatment of wastes (Chirumamilla et al., 2001). The enzymes may be of different organisms such as animals (glands), plant (seeds, fruits, exudates) and microorganism cultures. The latter make use of a total culture, extracting the enzyme from the culture medium (Coelho and Amaral, 2013). There is a growing recognition that enzymes may be used in many bioremediation processes such as the treatment of pollutants. The potential application of lignolytics enzymes has been the subject of extensive academic and industrial interest due to their ability to degrade a variety of toxic and recalcitrant pollutants.

The literature stresses the largest families of enzymes produced by fungi lignolíticos: -MnP manganese peroxidase (EC: 1.11.1), laccase - Lac (EC: 1.10.3.2) and lignin peroxidase - Lipstick (including EC 1.11.1.14), and the two most important processes in lignin degradation with a wide application in industries. Laccase is a copper containing enzyme active site iron while lignin peroxidase contains a prosthetic group. Lignin peroxidase is a heme protein having a high oxidation potential to oxidize phenolic and non-phenolic substrates. Laccase is an oxidase which catalyses the reduction of H_2 and O_2 to oxidize aromatic amines (D'Souza et al., 2006). As these enzymes do not have substrate specificity, they are employed in degradation, with application in chemical, food, agriculture, paper, textiles, cosmetics industries and in bioremediation treatments (Bonugli-Santos et al., 2010; Gomes et al., 2009; Sette et al., 2008; Pearce, 1997). Many microorganisms have been investigated in relation to their ability to produce enzymes, among which filamentous fungi are of great biotechnological interest. An important factor to be taken into account is that most fungi producing enzymes of this class are higher fungi of the phylum basidiomycote that are very difficult to cultivate in the laboratory. Among the fungi called lower, we can highlight the anamorphic of ascomycote and basidiomycote and zigomycote that has been reported in the literature as promising for enzyme production since coloados under ideal conditions (Miranda et al., 2013). One alternative for improving the production of these enzymes by other groups of fungi is induced mutation by radiation, one widely used technique that contains antibiotics and drugs derived from microorganisms. On changes in the molecules caused by ionizing radiation, directly or indirectly, studies have been developed in different fields of biology, including: creation of new plant varieties with improved characteristics; increased and improvement in food production through plant metabolism, animal and control or elimination of insects, fungi and / or bacteria (Okuno et al., 1982; Cardoso, 2006).

The biotechnological application of irradiation positively increases production of enzymes. This study is on the effect of gamma radiation on the enzymatic production of filamentous fungi isolated from water of Port Complex of Suape, Pernambuco.

MATERIALS AND METHODS

Microorganisms

In this work, the fungi, *Penicillium* sp., *Aspergillus terreus* and *Aspergillus awamori* used were obtained from seawater. 60 l of water (8 ° 24 '22.1' 'S 34 ° 57' 57 2 '' W) was collected from three random points: two points on the dike to capture sea water of Termope SA and a point on the river Ipojuca (next watershed in the collection point), the Suape Port Complex - Pernambuco.

Isolation of microorganisms

The seawater samples were processed through the manifold on nitrocellulose filter membranes with a porosity of 0.22 microns. After filtering these membranes with any condensate, they were transferred to Petri dishes with agar Sabouraud medium - BSA (10 g peptone, 40 g glucose and 15 g agar in 1 L of distilled water). The medium was prepared for use and after preparation, it was taken to autoclave. The whole procedure was performed in a laminar flow chamber. The Petri dishes were incubated at 30°C for up to 15 days and new isolations were performed every 15 days.

Preparation of samples for irradiation

In order to test the best technique of fungus inoculation, samples were prepared according to four different methods. With the previously fungi grown in Petri dishes, lyophilization (Figueiredo, 2001), scraping of spores (Carvalho et al., 2012), glass beads

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(Droce et al., 2013) and agar block (Kali et al., 2014) were performed.

Irradiator with cobalt source

In carrying out the irradiation, the fungus grown was placed individually in Petri dishes or flasks penicillin, wrapped in plastic wrap. The samples were subjected to radiation doses of 500, 1000, 1500 Gray (Gy) at a rate of 3,532 kGy / h in March 2014. In irradiating with cobalt-60, an MDS Nordion Gammacell 220 Excel source has also been prepared as a sample for this control procedure.

Enzyme assays

Inoculation of irradiated fungi

After irradiation the samples were inoculated, according to the technique used in each preparation. In the freeze drying techniques, scraping the spores from agar block, fungi were directly transferred to Erlenmeyer flask (500 ml) containing 200 ml of Sabouraud broth and incubated under stirring of 150 rpm for 72 h. For the glass bead technique, 5 ml of sterile distilled water was added to a Petri dish and the material was homogenized. After this process, an aliquot of 2 ml of spores was removed and transferred to Erlenmeyer flasks (500 mL). It was kept in the same conditions described above.

Assessment of enzyme activity

The mycelium of fungi grown on Sabouraud broth was filtered with liquid metabolite in sterile membrane of 0.22 micrometre (Merck Milipore®). The experimental procedure consisted of two stages: the first analysis was performed with samples of fungi in the control group (zero radiation) and the second was done with the samples after irradiation (500, 1,000 and 1,500 Gy). For each fungus and processing assays were performed in triplicate. To determine the enzymatic activity, the absorbance and appropriate length for each test wave was measured in a Thermo Scientific® spectrophotometer.

Activity of lignin peroxidase (LiP)

The LIP activity was determined by oxidation of veratryl alcohol according to the method of Gill and Arora (2001). The mixture is composed of 1 ml of buffer 125 mM sodium tartrate (pH 3.0), 500 ul 10 mM veratryl alcohol, 500 ul 2 mM hydrogen peroxide and 500 ul of enzyme extract. With the addition of the hydrogen peroxide reaction, the reading was done at 310 nm. One unit of each enzyme was defined as 1.0 micromol of product formed per minute under the assay conditions.

Activity of manganese peroxidase (MnP)

To quantify the manganese peroxidase (Bonugli-Santos et al., 2010), oxidation of phenol red (0.01% v / v) plus 500 µL of the enzyme extract, sodium lactate (0.25 M), bovine albumin (0.5% w / v) MnSO4 (2 mM) and H_2O_2 in citrate phosphate buffer (20 mM, pH 4.5) was measured. The reading was done at 610 nm.

Activity of laccase (Lac)

The activity of the laccase was determined using 2.2-azino-bis-etilbentiazolina (ABTS) as described by Gill and Arora (2001). In the mixture, 0.1 ml of sodium acetate buffer 0.1 M (pH 5.0) and 0.8 mL of a solution of ABTS were used at 0.03% (w / v), and 0.1 ml of the enzyme extract was the absorbance at 450 nm.

Statistical analysis

To determine if there was no statistically significant difference between enzyme production after radiation and the various inoculation techniques used, the student t test with significance level of $p < 0.05$ was used, with the help of statistic 8.0 software.

Assays for enzyme production by fermentation bioreactor

For the fungus with the highest enzyme production, an essay on biological reactor, brand Bioflo 110 (5 L) was carried out in tubes, containing two types of middle Sabourad (SAB) and liquid Bushnell Haas -BH compound 1 g KH_2PO_4, 1 g of K_2HPO_4, 1 g of NH_4NO_3, 0.2 g $MgSO_4.7H_2O$, 0.05 g $FeCl_3$, and 0.02 g $CaCl_2$ and $2H_2O$ (Atlas, 1995) milhocina- plus 40 mL/l. 3 L of SAB 250 blocks of agar (Ø 6 mm) was placed per liter, with microbial growth in irradiated best dose of the test vial, obtained in the enzymatic assay. In the second trial, 3 L Bioreactor middle BH (modified) was used, 250 blocks of agar (Ø 6mm) per liter, with irradiated microbial growth obtained in the enzymatic assay. The experiment was conducted at the temperature of 30°C, agitation of 150 rpm and pH 5.6. The trials were held for 72, 24 h where each was taken in a 10 ml aliquot of the sample for the evaluation of enzyme activity.

RESULTS AND DISCUSSION

Isolation of microorganisms

From the 140 strains of filamentous fungi isolated from water, 23 strains belong to the genus *Aspergillus* (16.43%), 20 strains, *Penicillium* (14,29) and the other 97 (69.28%), fungal species. The three selected specimens were *Penicillium* sp., *A. terreus* and *A. awamori,* having historical enzyme activity. Several authors agree with the results obtained in this work. Rajesh and Rai (2013) reported the high enzyme productivity of *Ventilago madraspatana* Gaertn fungus after being isolated from soil and indoor plants.

Enzyme assays

Assessment of enzyme activity

Activity of lignin peroxidase (LiP): The three fungi tested behaved differently in the production of lignin peroxidase enzyme under the three methods of inoculation employed (Figure 1). The best method of inoculation, scraping spores (ER) was used for *Penicillium*

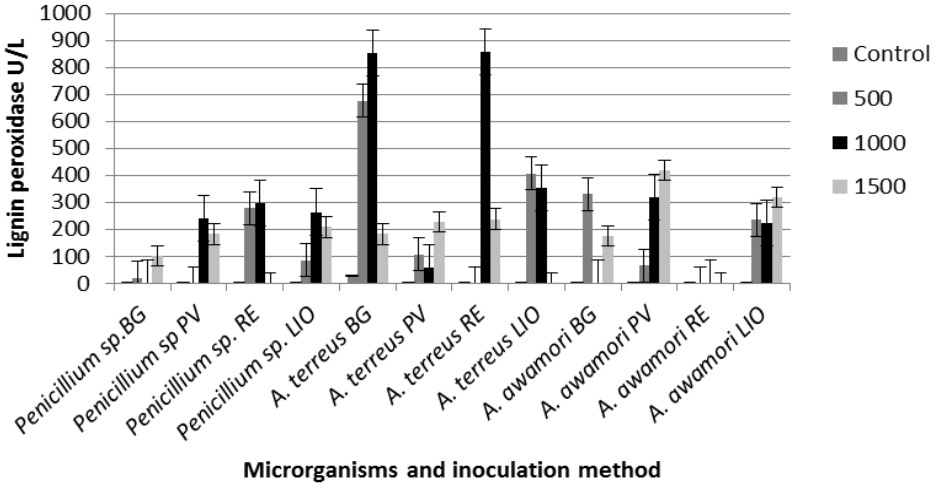

Figure 1. Values of enzyme assay for lignin peroxidase.

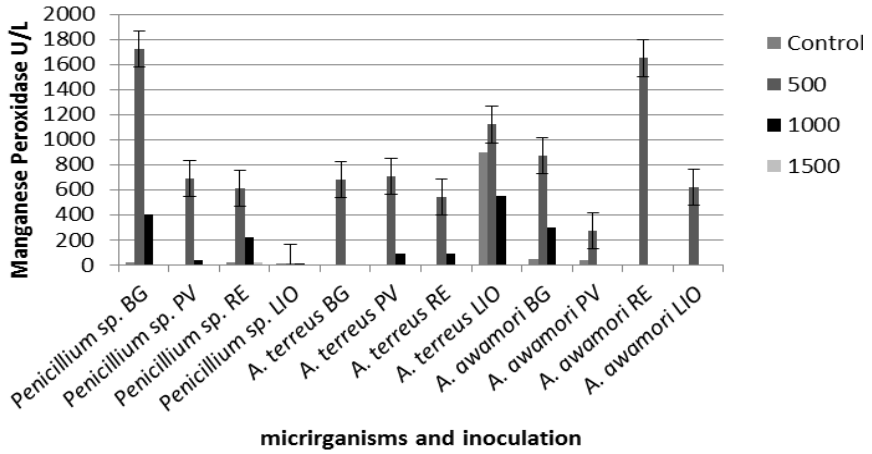

Figure 2. Values of the enzymatic assay for manganese peroxidase (MnP).

sp., where there was a production of 298 U/l enzyme at a dose of 1000 Gy. Although all the methods of inoculation favored the enzymatic production after irradiation of *Penicillium* sp. in all dose levels, there was a small production compared to other fungi studied. When the irradiated fungus was *A. terreus,* Block Agar (BG) and Scraping Spores (RE) methods had similar efficiency. The same yielded 852 to 858 U/L at 1000 Gy. Different from *Penicillium* sp., *A. terreus* had a higher significant production under two methodologies and in a dose of radiation. *A. awamori* had similar behavior with *Penicillium* sp.; they had similar enzyme production under the three irradiation methodologies (agar block, glass bead and lyophilization) in the three tested doses. The authors reported that genera of filamentous fungi have intermediate resistance to ionizing radiation, mainly *Aspergillus* and *Penicillium* genera (Rowley et al., 1978).

Filamentous fungi of the genus *Aspergillus* stand out as excellent producers of secondary metabolites of industrial and environmental interest, since they have a high rate of growth and a large thermotolerance, which favors studies of selection and production of high value-added bio-products (Berka et al., 1992; Ward et al., 2005; Lotfy et al., 2007; Mata-Gomez et al., 2009; Samson and Varga, 2009; Dhillon et al., 2012; Goswami et al., 2012; Singh and Mukhopadhyay, 2012; Chavan and Deshpande, 2013; Gopinath et al., 2013; Maldonato et al., 2014).

Activity of manganese peroxidase (MnP): When manganese peroxidase enzyme was studied, it was observed that all three fungi tested were producers (Figure 2). When the fungus *Penicillium* sp., was irradiated, agar block (BG) method of inoculation was the most effective, leading to the production of 1720U/L

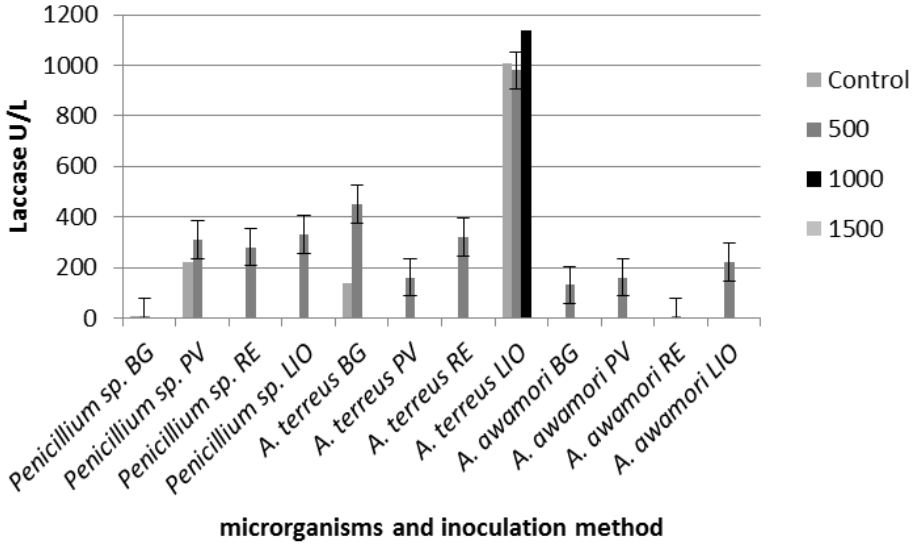

Figure 3. Values for laccase enzyme assay.

enzyme at 500 Gy. Although the fungus *Penicillium* sp. has produced manganese peroxidase with all methods of inoculation and in all doses of radiation, the values were insignificant compared to 500 Gy dose under block agar. When *A. terreus* was subjected to radiation, it was observed that freeze drying method was most effective in producing enzyme in all doses. *Penicillium* sp. at 500 Gy was able to induce the production of 1120 U/ L manganese peroxidase in the fungus *A. terreus*. The fungus, *A. awamori* showed that the best method of inoculation was scraping the spores, which led to the enzymatic production of 1650 U/L at 500 Gy dose. It can be observed at this step that 500 Gy dosages proved the most efficient for the three fungi tested, regardless of the inoculation method used. The growth capacity is lost at low doses; however, the culture age may considerably influence the radio sensitivity and therefore, experiments are conducted by different authors to determine the sensitivity to radiation in yeast and filamentous fungi. Yeasts are generally more resistant filamentous fungi (Diehl, 1995).

Treichel et al. (2010) describe that the genus *Aspergillus* excels due to its high biotechnological potential to produce high value-added bio-products, especially microbial enzymes. Colla et al. (2012) reported that the production of microbial enzymes by filamentous fungi is especially prized biotechnologically. This facilitates their recovery in the middle of production, and describes the genus *Aspergillus* as a good producer.

Activity of laccase (Lac): The production of laccase enzyme by fungi in three different doses of radiation is seen in Figure 3. The production of laccase enzyme by *Penicillium* sp. at 500 Gy was 330 U / L, but there was no production at 1000 and 1500 Gy. It was observed that for all inoculation methods described above and in all the doses, enzyme was produced by the fungus. *A. awamori* produced 220 U / L at a dose of 500 Gy, but there were no results at 1000 and 1500 Gy doses. It was observed that enzyme produced by this fungus was small in all the methodologies. *A. terreus* produced 980 U / L enzyme at 500 Gy dose and 1140 U / l at 1000 Gy; these were the highest enzyme production, but there was no production at 1500 Gy. It was observed that the fungus at 500 and 1000 Gy doses was efficient when freeze-drying (IOL) method was used. Since there are few studies on using fungi for the production of irradiated metabolites, the closest microorganisms reported are lichens which when subjected to gamma or ultraviolet radiation tend to produce larger amounts of phenols; therefore, they act as sunscreens and foto indutores or protect the interior of the stem while preserving their physiology (Seaward, 1977). The same thing may have occurred with the micro-organism under study.

In *Cladonia salzmannii* found in soil leachates, increased production of acid barbático (FAB) occurred when they were irradiated at 5 Gy (152,9 mg bar / stem mg) and 10 Gy (86,8 mg bar / mg stem). Here, higher concentrations of these compounds were detected. The concentration of BAR in samples subjected to 60 and 100 Gy doses, respectively, was not higher than that of the control. It can be suggested that, doses from 60 Gy negatively influence biosynthesis liquênicos compounds analyzed, inhibiting, with increasing doses of gamma radiation, the production of BAR (Melo, 2011). These results are similar to that of this work, where 500 Gy dose

applied gave better results, and 1500 Gy dose negatively influenced enzyme production. The production of atranorina (ATR) by *Cladonia verticillaris* not exposed to gamma radiation was 0.024 mg.mL^{-1}. Later, the TR range of exposure of the substance was approximately 27 min. The output 10 for samples irradiated at 1,000 and 10,000 Gy was 0.15, 0.06 and 0.20 mg.mL^{-1}, respectively (Silva, 2011). In this experiment, the higher dose of gamma radiation was the one with higher production. Another isolated from *Cladonia verticillaris* production of ATR for the samples irradiated at 10, 1000 and 10,000 Gy was 0.07, 0.03 and 0.08 mg.mL^{-1}, respectively. Thus, there is a greater production with an irradiated sample at 10,000 Gy. This change is probably due to changes in the chemical composition of the substance blocked in the metabolic pathways by radiation (Silva et al., 2010).

The data concerning the enzymatic activity of fungi tested in this work are still insufficient. That is why it is important to continue and deepen the studies, as well as to implement other complementary techniques. However, the present study demonstrates that there is an improvement in enzyme production in filamentous fungi, especially at 500 Gy, which allows further development of this metabolite.

Statistical analyses

According to the T test, there were statistically significant differences in enzyme production at 500 Gy dose. Comparing the control treatment with radiation dose of 500 Gy, lignin was p = 0.007832 and manganese was P = 0.000155. To control a1000 and 1500 Gy, significant differences of p = 0.001881 and p = 0.000627, respectively were observed for lignin. Comparing the doses from 500 to 1000 MN showed only a difference of p = 0.000032; when comparing the laccase of 500 with 1500, we obtained p = 0.000067 and P = 0.000001, respectively for manganese. The comparison between 1000 and 1500 revealed only a difference for manganese peroxidase with p = 0.008845.

According to the results obtained in this work it can be inferred that the three proposed dosages improved the enzymes produced by the selected fungus, according to the proposed objective. It is of utmost importance for industries which use strains to produce metabolites of industrial interest to make the process cheap. There was an improvement in the production of three enzymes of industrial interest when the yeast was subjected to low doses of radiation.

The results indicate that the different techniques in which the inoculation was subjected to fungi caused no abnormalities (statistically significant differences at the level of p = 0.05), and any of such techniques can be repeated for the reproduction of enzyme. The best technique for reproduction is agar (BG) block, for it is easy and economically important for use in scientific research and industrial production criteria.

Assays for enzyme production by fermentation bioreactor

In an attempt to reduce the environmental impacts generated by agro-industrial activities, government agencies and industries are seeking new environmental policy. And research shows that waste still contains a lot of organic matter and other by-products, which can be used as a source for the generation of other products, such as animal feed, nutrients for micro-organisms in various processes (Pelizer et al., 2007). The corn steep liquor is a byproduct of processing corn. Corn steep liquor contains a lot of nitrogen, amino acids and other nutrients, and is used primarily as a food supplement for manufacturing feed for poultry and ruminants. Some studies are being developed for fermentation processes that accrue as a source of nutrients for micro-organisms (Amartey and Leung, 2000). Based on the results obtained from the enzyme production and the statistical analysis, one bioreactor test of this process was done on two types of media. *Penicillium* sp was irradiated at 500 Gy and led to the production of the best enzymes- laccase and manganese peroxidase. For the analysis of lignin in the middle SAB enzyme Bushnell Haas + corn steep liquor, the highest values obtained were 108 and 560 U/L (Figure 4).

Recently, soil fungi have been examined for their ability to degrade hydrocarbons and producing ligninolytic enzymes (Silva et al., 2009). Lignin peroxidase has been used to mineralize a range of recalcitrant aromatic compounds such as hydrocarbons and dyes (Wesenberg et al., 2003). Clement et al. (2001) studied the degradation of hydrocarbons by thirteen deuteromycete ligninolytic fungi and found that the degradation degree varies with the ligninolytic enzymes. Moreira (2006) reported in his work that *Psilocybe castanella* produced a high ligninolytic activity, probably in response to the concentration of the organic pollutant hexachlorobenzene. Anastasi et al. (2009) reported a lipase production of 19 U/L by Basidiomycetes. Compared to that produced in this work, a better result was obtained. Eight fungal strains used in bioremediation were isolated from agricultural soils cultivated with rice. The major enzyme activities detected were related to the production of lignin peroxidase. The maximum detected level was 6079 U/L^{-1} (strain P11SA4F), followed by 3,332 U/L^{-1} (strain P11SA4F). None of the tested fungi can be compared to LiP production (18,851U L^{-1}) by *Ganoderma* sp strain GASI3.4, used as the control (Silva et al., 2004). The fungus *Penicillium commune* produced 2,500 U/L lignin (Baptista et al., 2011). Lignin peroxidase was produced thus: *Paecilomyces* sp. produced 94 U/L ± 9

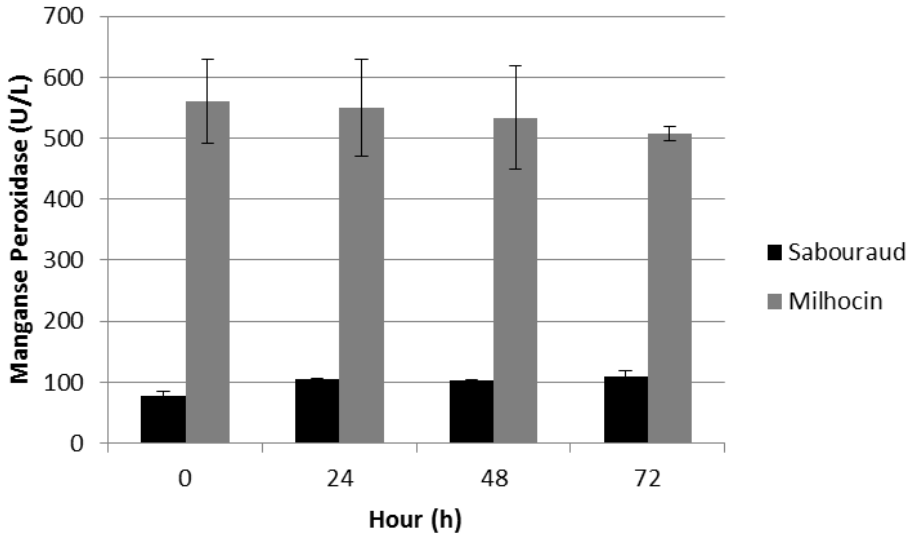

Figure 4. Test bioreactor for lignin.

lignin peroxidase; *Penicillium* sp., 100 U/L ± 22; *Aspergillus* sp., 100 U/L 14 ±; and *Penicillium* sp., 144 U/L ± 13 (Maciel, 2010). Gomes et al. (2009), in decolourisation of stains, obtained 9 U/mL of lignin peroxidase after five weeks of incubation. Anastasi et al. (2009), in his study, observed a LiP production of around 19 U / L by Basidiomycetes. Thus, the results obtained for *Penicillium* sp., and *Aspergillus* sp. proved to be superior to that of the literature. Of all the tested fungi grown in pure cultures that produce lignin peroxidase, *P. commune* had higher enzyme production, reaching 2,515 U/L (Arruda, 2011). The maximum activity of LiP by Basidiomycetes found was 20 mmol/L at 24 h incubation in the work cited. The LiP activity detected was 3.58 U/L, greater than the activity detected by Zhao et.al., (1996) and Arora et.al., (2002), where they found only 0.173 U/g and 1 U/ml, respectively, from other white rot fungi of the wood.

For manganese enzyme (Figure 5), from the initial period to the middle in SAB medium containing corn steep liquor, good results were obtained, close to 600 U/L for 24 h period. This was almost the same for both media production, but we observed a slightly better outcome in the SAB; at the middle in SAB, there was a low level of enzyme production (40 U/L) for 48 h; the medium containing corn steep liquor had a great result of 550 U/L. For 72 h, enzyme (4 U/L) was produced only in corn steep liquor media. The results were directly proportional to the increase in hours. Thus, the starting time (72 h) stimulated an increased enzyme production of 300 U/L in the bioreactor milhocin medium; for lignin peroxidase and manganese, best result found in the medium containing corn steep liquor was 555 U/L.

Regina et al. (2009) obtained maximum values of MnP activity around 1400 U/L for *Lentinus edodes* grown in liquid-based infusion of cassava bagasse and dextrose medium. However, the same authors also observed the influence of the substrate on the expression of the enzyme; with the infusion of crushed cane sugar and dextrose, the maximum value was obtained at 400 U/L. Betini (2006) demonstrated that the fungus, *Aspergillus niger,* xylanase produced a concentration higher than 30% when cultured in a medium containing wheat bran as only carbon source compared to medium that also contains corn cobs. This demonstrates that supplementing with cob meal appears to satisfactorily answer the enzyme production by the fungi; although Kadowaki et al. (1997), in their studies, obtained maximum production of xylanase by *A. tamarii* when it was grown in medium supplemented with a high concentration of solid waste from *Zea mays* (corn cob). *Gloeophylum byrsina* and *Coriolopsis striatum* are excellent producers of MnP and produced 67.1 U/ml (21 days bagasse) and 590.3 U/ml (28 days in rice straw), respectively compared to those of Nuske et al., (2002), where *Nematoloma frowardii* produced 1.5 U/ml after 11 days of fermentation in wood chips as a substrate. *Phanerochaete chrysosporium* fungus was widely studied for its ability to produce ligninases (Fujian et al., 2001). The highlight in the production of manganese peroxidase was: 60.0 U/L ± 8 produced by *Penicillium* sp, about 56 U / L ± 6 generated by *Curvularia lunata* and 51 U/L ± 4 by *Paecilomyces* sp. (Maciel et al., 2010). Similar results were obtained by Gomes et al. (2009), who decolorized dyes using rice as a substrate and obtained 0.6 U/mL of peroxidase-Mn. However, better results were observed by Anastasi et al. (2009) in their degradation tests using basidiomycetes that produced about 124 U/Ll MnP.

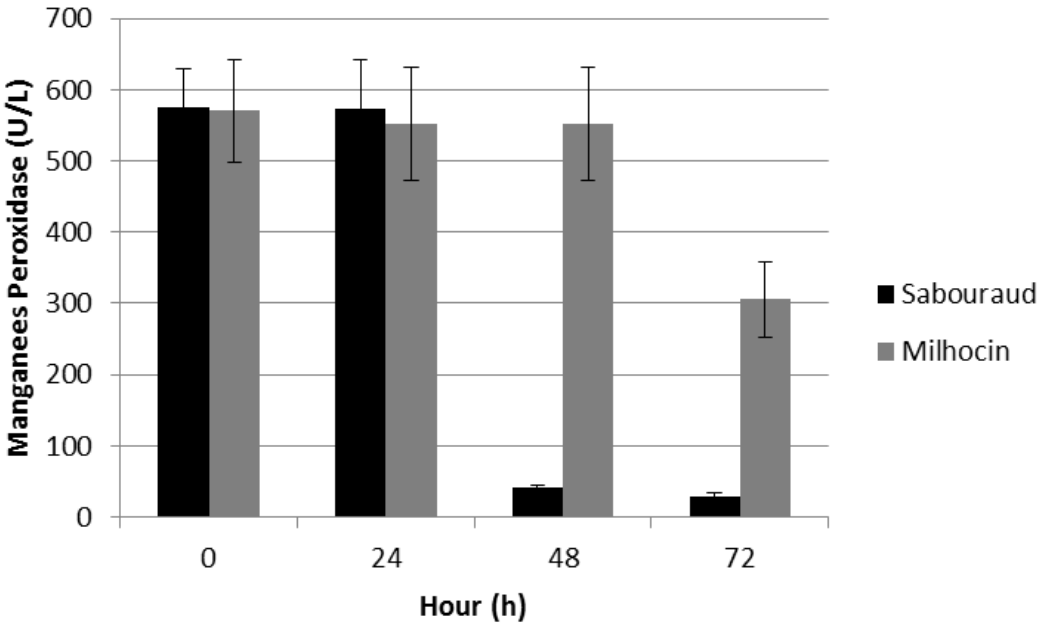

Figure 5. Test bioreactor for manganese peroxidase.

For the enzymatic activity of laccase (Figure 6) at the initial period, sample with the greater amount of corn steep liquor produced 650 U/L enzyme than at the midle in SAB where 460 U/L enzyme was produced for 24 h; at the middle, enzyme production (300 U/L) decreased in SAB and in the medium containing corn steep liquor (559 U/L enzyme) for 48 h. The medium containing corn steep liquor presents a slight increase over the 24 h period, with 620 U/L enzymatic production. For 72 h, in the medium containing the milhocin, there was enzymatic production of 610 U/L. The enzyme laccase showed the best results compared to the other enzymes produced in this work.

It is possible to observe that the condition where supplementation of corn steep liquor was found has the best enzyme production. One of several factors that can influence the growth of a microorganism is the nature of the culture medium. The difference in composition of media is a factor that could change the metabolites produced. *Penicillium* sp. (290 U/L ± 28) and *C. lunata* (210 U/L ± 17) stand out statistically in the production of laccase enzyme compared to other fungi (Maciel et al., 2010). For Quarantino et al. (2008), laccase production by *Panus trigrinus* ranged from 0.024 to 2.04 U/mL, confirming the results obtained in this work. Amid Sabouraud broth plus diesel oil, glucose was used as control and diesel oil as inducer of enzyme activity, where laccase activities range from 4.35 to 4.62 U/L, highlighting the highest production for *Cunninghamella echinulata* and fungi Penicillium commune with 4.62 U/L for both (Baptista et al., 2011). The biological functions of laccase in micro-organisms are still not very clear. In fungi, there are reports about its involvement in rapid cell growth, sporulation (Gianfreda et al., 1999) and degradation of lignin (Eggert et al., 1996). The largest production of laccase was in Sabouraud liquid medium by the fungus Penicillium commune, reaching 1,947 U/L (Baptista et al., 2011)

According to Rothschild et al. (2002), the activity of lipase and laccase has been reported in some white rot fungi. Using diesel oil as substrate resulted in the highest value for *C. echinulata*, 2,594 U/L. The results obtained are comparable or higher than those found by Narkhede and Vidhale (2005) who observed the production of polyphenol oxidases by *C. lunata* isolated from industrial effluent. Paecilomyces species have been reported in literature as being able to degrade substrates, lignolíticos, with production of polyphenol (Kluczek-Turpeinem et al., 2003). According to Kluczek-Turpeinem et al. (2007), the secretion of lignin degrading enzymes is a key step for the metabolism of carbon per *Paecilomyces* spp. representing a significant potential for detecting the expression levels of these enzymes.

Conclusion

The use of gamma radiation increases enzyme production by filamentous fungi. These results obtained in the research can prove to be useful in areas such as biotechnology, and thus add further information to the topic that is still pioneering.

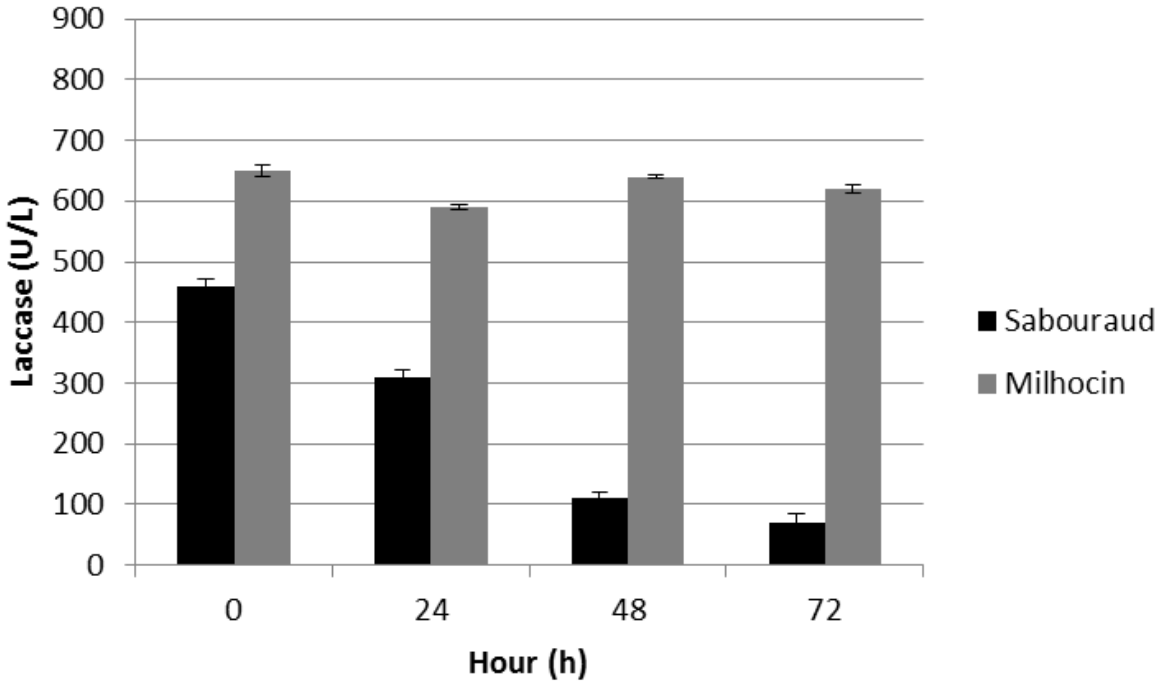

Figure 6. Test bioreactor for laccase.

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