



MICROBIOLOGY

Bioprospection of ligninolytic enzymes from marine origin filamentous fungi

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Abstract: Fungi are excellent producers of extracellular enzymes. Therefore, the present study aimed to investigate the screening of marine fungi, which are laccase and manganese peroxidase potential producers, in solid fermentation for future applications in bioremediation processes of contaminated sites. For this purpose, two-level factorial planning was adopted, using time (6 and 15 days) and the absence or presence of oil (0 and 1%) as factors. The semi-quantitative evaluation was carried out by calculating radial growth, enzyme activity and enzyme index by measuring phenol red or syringaldazine oxidation halo. The results showed that all the studied strains showed a positive result for manganese peroxidase production, with an enzymatic activity in solid medium less than 0.61, indicating a strongly positive activity. Through the enzyme index, the study also showed prominence for *Penicillium* sp. strains, with values > 2. The enzyme index increase in oil presence and the inexpressive use of the genera studied for ligninolytic enzymes production from crude oil demonstrated these data importance for fermentative processes optimization. Considering the ability of these strains to develop into recalcitrant compounds and the potential for manganese peroxidase production, they are indicated for exploitation in various bioremediation technologies, as well as other biotechnological applications.

Key words: bioremediation, enzymatic activity, manganese peroxidase, mycoremediation.

INTRODUCTION

The production of enzymes from microorganisms exposes great biotechnological, industrial and environmental interest due to its wide catalytic activities variety, large-scale production possibility, adaptation ease, renewability capacity and propensity for socioeconomic and environmental merits (Behbudi et al. 2021). Therefore, ligninolytic enzymes have a background for white biotechnology, with great potential for generating bio-products with high added value from natural biomass, following the green agenda (Bilal & Iqbal 2020).

Ligninolytic enzymes (LEs) are made up of three main representatives: lignin peroxidase (LiP, EC 1.11.1.14), manganese peroxidase (MnP, EC 1.11.1.13) and laccase (Lac, EC 1.10.3.2), along with others accessory enzymes (Wong 2009) and have the function of catalyzing modifications or degrading lignin into less complex molecules (Asemoloye et al. 2020). These enzymes have great versatility that covers a wide spectrum of use in bioremediation, and it is pointed out its complex recalcitrant compounds degradation and mineralization capacity, such as petroleum compounds and their derivatives (Asemoloye et al. 2020), pesticides (Zeng et al. 2017), drugs, such as endocrine disrupters (Eldridge et al. 2017)

and polycyclic non-steroidal anti-inflammatory drugs (Bankole et al. 2021), synthetic fragrances (Vallecillos et al. 2017), lignocellulosic residues (Kumar & Chandra 2020) textile effluent dyes (Rather et al. 2018) and also allows biocatalytic plastic recycling (Wei & Zimmermann 2017), bleaching paper (Nathan et al. 2018) and biosensors production (Sidwaba et al. 2019) among others. Most residues cited above have in their composition compounds that can cause adverse effects to the natural environment and to humans, among them polycyclic aromatic hydrocarbons (PAHs), formed by two or more condensed aromatic rings and responsible for several biological effects, such as high toxicity, mutagenicity and carcinogenic potential (Kadri et al. 2017).

The bioremediation use as a strategy for affected areas recovery has advantages in being an ecologically correct and cheaper technique when compared to chemical and physical methods of remediation (Kurniati et al. 2014). In recent years, interest in using fungi ligninolytic system as bioremediation agents has grown exponentially (Rao et al. 2014). This interest is mainly due to its oxidative efficiency, sustainable character, its low specificity to the substrate, feasibility of improving its production, stability and activity, and the possibility of immobilizing them when the use of microorganisms is limited (Ogola et al. 2015).

Many *Aspergillus* sp. and *Penicillium* sp. fungi species are reported in literature as efficient in natural environments contaminated by organic pollutants bioremediation through extracellular production of LEs (Li et al. 2020). Marine environments are an extraordinary source of microorganisms diversity capable of adapting to extreme conditions, therefore their enzymes are very attractive in unusual bioprocesses (Theerachat et al. 2019). Thus, marine origin fungi have great potential for industrial application,

once they are capable of producing enzymes with different physiological characteristics, such as tolerance to high rates of salinity and pH, high pressures, thermostability, psychrotolerance, thermostability and barophilicity (Bonugli-Santos et al. 2015).

The demand for screening studies of new enzymes is a need that extends from today, in order to improve more competitive and sustainable production processes (Adrio & Demain 2014). It is known that enzymes have different characteristics depending on the species, strains and cultivation conditions, therefore, although they have been extensively isolated and characterized from different natural sources, enzymes production system is a limitation for further large scale and reduced cost exploration (Sharma et al. 2018). In this sense, it is understood that for industrial processes development aiming enzymes production, initially the isolation and selection of microorganisms potentially producing enzymes with biotechnological interest is required, making bioprospecting studies fundamental (Paludo et al. 2019).

The use of simple methods can be effective in microorganisms characterization and configure a quick and useful screening when evaluating individual fungi (Hankin & Anagnostakis 1975). The direct correlation of radial growth diffusion and oxidation halo formed in solid culture medium can be used to determine the activity of extracellular enzymes (Ceska 1971). Thus, the selection of undesirable compound degrading fungi through their enzymatic activity represents a viable evaluation strategy for obtaining promising biotechnology industry microorganisms. (Lin et al. 1991). Therefore, the present work aims to screen marine origin filamentous fungi that are laccase and manganese peroxidase potential producers in solid fermentation for

future bioremediation processes applications in hydrocarbon contaminated sites.

MATERIALS AND METHODS

Strains

Five strains of filamentous fungi isolated from mangrove sediment in the Todos os Santos Bay, Brazil were obtained from the library of the Centro de Excelência em Geoquímica do Petróleo (LEPETRO) at the Federal University of Bahia. Data derived from phylogenetic analyzes (Supplementary Material – Figure S1 and Table S1) identified these fungi as *Aspergillus* sp. (R16), *Penicilium* sp. 1 (S40), *Penicilium* sp. 2 (R31), *Penicilium* sp. 1 (R26) and *Byssochlamys* sp. (N89). These microorganisms were selected for their ability to tolerate / degrade petroleum hydrocarbons (Lima et al. 2017, 2018) and therefore constitute a mixed microbial consortium protected by the patent with deposit number BR 10 2021 002341 4 (Lima et al. 2021), at the Instituto Nacional de Propriedade Industrial (INPI) and endorsed at the National Biotechnology Information Center (NCBI) under access numbers MW855899, MW855902, MW855901, MW855900, MW855903, respectively. They were grown on Sabouraud Dextrose Agar (SDA) (Kasvi®, Brazil) and maintained at 4 ° C for further analysis.

Plaque screening test

The production capacity of lignolytic enzymes was determined from the oxidation of specific compounds present in the culture medium (Agrawal et al. 2017), where the 0.1% (m / v) syringaldazine solution was used for Lacase (Lac) and the 2 mM MnSO₄ solution and 0.1% (w / v) phenol red (Sigma-Aldrich®) for Manganese peroxidase (MnP) according to guidelines adapted from the method described by Szklarz et al. (1989) and Kuwahara et al. (1984),

respectively. One disc (1 cm Ø) of pure colonies from each fungus was inoculated in the center of the Petri dish containing the Bushnell Haas medium (BH) (Difco®, United States) plus 1% (v / v) of oil from the Recôncavo basin sterilized in UV for 15 min, for condition I and in the culture medium Sabouraud Dextrose Agar (SDA) (Kasvi®, Brazil) for condition II. Subsequently, the strains were incubated at 30 °C and changes were observed for 15 days.

Enzyme index calculation

The identification of positive enzymatic reactions occurred through the formation of a discoloration halo around the colonies. The semi-quantitative determination of the enzymatic potential was evaluated by measuring the diameter of the halo produced and the radial growth of the colony with a millimeter rule. The data were initially submitted to the calculation of the enzymatic index (IE), which reflects the ratio of the diameter of the halo to the diameter of the colony (Hankin & Anagnostakis 1975) and subsequently the enzymatic activity (Pz) which corresponds to the ratio between the mean value the growth zone and the total growth zone plus oxidation of each strain (Price et al. 1982). Pz is classified and coded from the values obtained, where: it is considered negative when equal to 1, Pz = class 1; positive when <1> 0.64, Pz = class 2 and strongly positive when <0.64, Pz = class 3 (Queiroz & De Souza 2020).

Experimental design

The factorial design adopted was 2², with two replicates totaling 4 experiments for each strain. Two levels related to the lowest (-1) and highest (1) were adopted, where 6 and 15 days are related to time (factor 1) and 0 and 1 are for the absence of oil (factor 2). These experiments were carried out to predict better conditions for the fungi's ligninolytic enzyme index as a function of time

and the presence or absence of oil in the culture medium.

Statistical Analysis

The experiment was conducted with random sampling, with repetitions for each strain and cultivation condition. The data obtained were analyzed using the Minitab software version 20.2.0.0, where factor planning and normality and homogeneity tests of variance were applied, considering a value of $p \leq 0.05$.

RESULTS AND DISCUSSION

As shown in figure 1, the strains when grown in BH medium corrected with manganese sulfate and phenol red became colorless, showing complete degradation of phenol red, in SDA medium the reaction was reversed from colorless to red in different shades. On the other hand, there was no change in culture media color which syringaldazine was used as a standard substrate (data not shown) for Lac. Therefore, the results obtained by observing the discoloration halo,

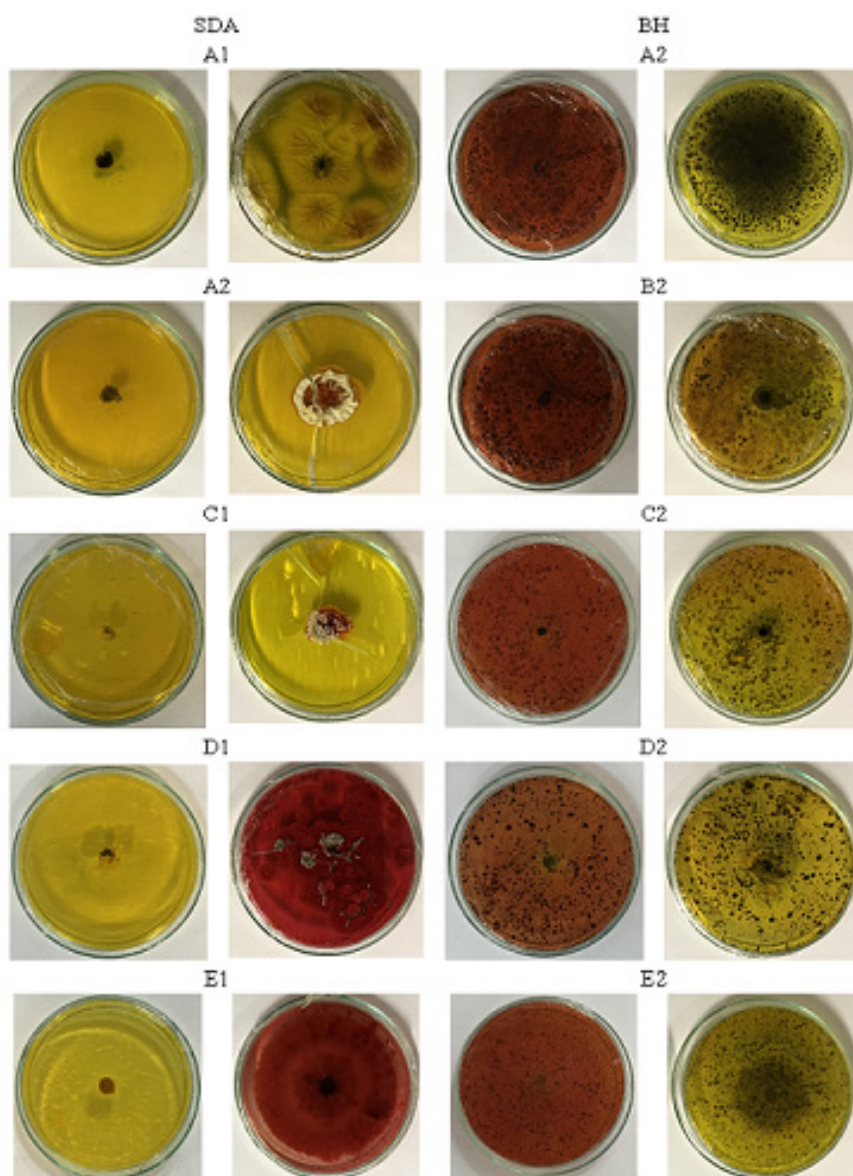


Figure 1. Phenol red discoloration halo seen from back by fungal strains in SDA medium (1) and BH medium (2) in 1 day and after 15 days of incubation. a) *Aspergillus* sp. b) *Penicilium* sp. 1 c) *Penicilium* sp. 2 d) *Penicilium* sp. 1(R26) e) *Byssoschlamys* sp.

shown in Fig. 1, demonstrated that *Aspergillus* sp., *Penicilium* sp. 1, *Penicilium* sp. 2, *Penicilium* sp. 1(R26). and *Byssoschlamys* sp. has the capacity to produce manganese peroxidase in a solid culture medium, however it did not show activity for Lacase. The same result was found by Vipotnik et al. (2021), where it was not possible to detect Lac for *P. chrysogenum* in 2 weeks of incubation in solid fermentation under the influence of pH 7.

Lignolytic enzymes production is influenced by several factors, such as carbon and nitrogen source, growth conditions and the effect of aromatic compounds (Schneider et al. 2018), as well as genetic, physiological or ecological specificities of each fungus (Elisashvili et al. 2010). It is known that catalytic activity requires the presence of cofactors, such as metals and coenzymes, as well as an ideal pH range, so their absence can affect the production and activity of enzymes (Coelho et al. 2008). In this sense, it can be inferred that, probably, the cultivation conditions were not satisfactory for laccase production, since the ideal pH for production in *P. cyclopium* and *P. digitatum* was considered acid close to 5 and temperature of 25 ° C (El-Shora et al. 2008), different from what was carried out in this study, pH 7 and 6 ± 0.2 at 30 ° C for BH and SDA, respectively. On the other hand, the results found in Kumar et al. (2016) are divergent, since *P. martensii* obtained optimum laccase production conditions at 35° C in a pH 7, indicating optimum conditions variation for each strain. As for the genus *Aspergillus*, *A. flavus* Lac production was found from in solid medium, both at acidic pH and at pH close to neutrality (pH from 3.5 to 7.5) and in temperature variations of 20° C at 35° C (Aftab et al. 2018). Notwithstanding were the results of Hu et al. (2011), where he obtained enzymatic activity for Lac in *A. niger* and *A. oryzae*, both in individual culture and in co-cultivation at

25° C or 30° C in a pH 6.0. One of the major differences between the works mentioned and the current one is in substrate, for this reason we can infer that, although it was possible to observe the production of Lac by other authors at pH 7, the optimal pH also depends on the substrate used (Fukushima & Kirk 1995). In this sense, once most fungi laccases are preferably produced at a pH close to 3 using 2,2'-Azino-Bis (ABTS) as a substrate (Kumar et al. 2016), it is believed that syringaldazine can also be similar and therefore resulted in the non-detection of Lac in the conditions provided. Furthermore, pH in acidic conditions can favor the development of filamentous fungi and assist hydrocarbons degradation (Maciel et al. 2010). Regarding *Byssoschlamys* genus, although it is widely used in bioremediation strategies (Mann et al. 2010, Hechmi et al. 2016) some species, such as *B. nivea* are not able to produce the enzyme laccase or lignin peroxidases (Bosso et al. 2015), which corroborates our findings.

Laccase has a limited effect on bioremediation due to its specificity for lignin phenolic units and thus some substrates cannot be directly oxidized by Lac (Theerachat et al. 2019), in these cases the limitation can be overcome with the addition of mediators, as well as some other enzymes, such as: feruloyl esterase, lipases, aryl-alcohol oxidase, catechol 2, 3-dioxygenase, quinone reductases (Kumar & Chandra 2020). Thus, it is necessary to optimize the adaptable substrate condition to these fungi, as well as to test different redox mediators concentrations, such as 2,20-azino-bis and p-cumárico acid, 1-hydroxybenzotriazol (Bankole et al. 2021) or the inclusion of inductors, such as copper sulfate (De Salas et al. 2019, Ferraroni et al. 2017), ethanol, methanol, isopropanol, acetone, tween 80, glycerin, glucose, triton X, sulfate iron, calcium chloride, sodium nitrate

(Aftab et al. 2018), in order to improve the target enzyme activity.

Through figure 1 it is possible to observe the culture media color change around the strains colony diameter after 15 days of incubation. Manganese peroxidase is a manganese-dependent enzyme, which, from the manganese oxidation enables the subsequent oxidation of phenolic compounds (Glenn et al. 1986). Thus, this enzyme synthesis is indicated through the phenol red oxidation and subsequent color change, as shown in figure 1. The results showed the enzyme presence in all fungi in a culture medium with 1% oil. This enzyme production in response to crude oil increase in *A. niger* corroborates the data found in Asemoloye et al. (2018), which acquired 70 U.mL⁻¹ of MnP when produced in 5% of oil. Similar results were observed in Maciel et al. (2010), using diesel oil as a substrate and reached a production of 60 U.L and 7 U.L for MnP from *Penicillium* sp. and *A. tamarii*, respectively. For *Byssochlamys* genus, 0.17 IU.mL of MnP production was also observed, according to M'barek et al. (2019).

Among fungal peroxidases, MnP has great ecological importance, mainly due its plant biomass conversion and recalcitrant compounds degradation (Kellner et al. 2014). This enzyme is extensively explored biotechnologically because it mediates textile dyes biocatalysis reactions (Zhang et al. 2020), in addition to presenting high petroleum hydrocarbons degradation rates (Becarelli et al. 2019). Therefore, the cited fungi demonstrated ability to secrete MnP, which gives them great potential for environmental and industrial applications, such as pulp and paper industries for bio-bleaching processes, pulp production and effluent purification (Saldarriaga-Hernández et al. 2020) or in the food industry, producing natural aromatic flavors (Li et al. 2015). Furthermore, these enzymes can play a vital role in biofuels

production and other biochemicals, since they oxidatively depolymerize lignin and leave the polysaccharides intact (Maciel & Ribeiro 2010).

Table I describes the enzyme index and the enzyme activity found for manganese peroxidase of studied fungi at the end of 15 days of incubation. The enzymatic activity of MnP varied between 0.24 and 0.61, grouping all strains as class 3. Since the Pz is less than 0.64, the strains are classified with strongly positive activity, which is, the lower the value of Pz, the greater the potential for production of the enzyme and therefore should be considered excellent producers of the enzyme in question.

Since *Aspergillus* sp. showed enzymatic activity of 0.61 and 0.37 in SDA and BH media, respectively, it is believed that it provides favorable characteristics for MnP production and, consequently, bioproducts production. No reports were found in the literature regarding Pz, however it is known that *Aspergillus* sp. are well known for secreting large amounts of enzymes (Hernández-Martínez et al. 2011) and for this reason they are often mentioned in several studies as efficient producers of lignolytic complex enzymes, among them the work of Reis et al. (2019) where *A. niger* is cited as a viable alternative production of multiple enzymes, including MnP and Lac. In addition, the *A. niger* strain can be adopted as a biodegradable agent for complex compounds, such as effluent discoloration composed of reactive yellow and reactive red dye, reaching 98.62% and 92.42% removal, respectively, as described by Salem et al. (2019), and as an alternative, *A. niger* it can also biodegrade the Congo Red dye, reaching a discoloration rate of 97% as a result ligninolytic enzymes action, where MnP plays a crucial role in discoloration (Asses et al. 2018).

All three *Penicillium* strains showed favorable results to production MnP, with increasing values of 0.31, 0.50, 0.53 on SDA

Table I. Average and standard deviation of the enzyme index and enzymatic activity of strains for MnP in solid medium in 15 days of incubation.

Microorganism	Enzymatic Index (IE)		Enzymatic Activity (Pz)		Radial diffusion (cm)	
	SDA	BH	SDA	BH	SDA	BH
<i>Aspergillus</i> sp.	0.64 ± 0.03	1.64 ± 0.21	0.61 ± 0.01	0.37 ± 0.03	9.00 ± 0.00	4.85 ± 0.21
<i>Penicillium</i> sp. 1	0.97 ± 0.03	1.85 ± 0.27	0.50 ± 0.03	0.35 ± 0.00	3.50 ± 0.28	2.00 ± 0.00
<i>Penicillium</i> sp. 2	0.88 ± 0.17	3.15 ± 1.32	0.53 ± 0.05	0.25 ± 0.08	2.55 ± 0.35	2.50 ± 0.42
<i>Penicillium</i> sp.1(R26)	2.21 ± 0.22	3.18 ± 0.39	0.31 ± 0.02	0.24 ± 0.02	4.10 ± 0.42	3.00 ± 0.14
<i>Byssoschlamys</i> sp.	1.15 ± 0.02	1.53 ± 0.14	0.46 ± 0.00	0.42 ± 0.02	7.80 ± 0.14	5.90 ± 0.56

media and 0.24, 0.25, 0.35 on BH media. Although the strains potential mentioned, in terms of volume, is not known, many *Penicillium* fungi have a great consolidated biotechnological role to degrade different compounds, as well as in Govarthanan et al. (2017) work, where he records in his study with *Penicillium* sp. obtaining 1.0492 U of crude MnP enzyme in the degradation process of decane, butylbenzene, dodecane, naphthalene, acenaphtene, octane, ethylbenzene and benzo [a] pyrene. In contrast, Chen et al. (2019) obtained a production range of 4.68–23.31 U mL⁻¹ of MnP from *P. simplicissimum* on triphenylmethane discoloration. Thus, this statement adds relevance to the isolates studied for the degradation of undesirable aromatic compounds.

Although there are few reports in the literature regarding the production of ligninolytic enzymes by *Byssoschlamys* strains, the genus is mentioned as adapted for development in environments contaminated by hydrocarbons (Radwan et al. 2018) and is often pointed out as responsible for corrosion of metals (Blaney 2007). However, *B. nivea* is mentioned as efficient in mycoremediation process of sites contaminated by aromatic compounds, such as pentachlorophenol (Hechmi et al. 2016)

and metabolizing biodiesel and petroleum diesel (Ye et al. 2017). Representatives of this genus also have genes involved in aromatic compounds and n-alkanes degradation, including cytochrome P450 alkane hydroxylase, cytochrome P450 monooxygenase, aromatic ring-opening dioxygenase, salicylate hydroxylase, 2-haloacetaldoxygenase, benzyl alcohol dehydrogenase, benzoate 4- monooxygenase and dimethylsulfide monooxygenase (Radwan et al. 2018). White rot fungi ability to degrade aromatic compounds is attributed to MnP and Lac activity, which is often focused on Basidiomycota, among them *Aspergillus* and *Penicillium*, however Ascomycota representatives such as *B. nivea* are neglected (Hechmi et al. 2016). Therefore, the data from this study may be a stimulus for further investigations regarding these enzymes production from *Byssoschlamys* genus and the strain exploration for bio products productions for white biotechnology.

Enzyme activity index is one of the most used semi-quantitative parameters to evaluate the production of enzymes by microorganisms in a solid medium (Rezende et al. 2013). Based on this assumption, all the studied fungi can be considered manganese peroxidase producers, according to the data shown in Table I. However,

Aspergillus sp. and *Byssochlamys* sp. strains showed activity below 2 in SDA and BH medium, on the other hand, *Penicillium* sp.1 (R26) showed an enzyme index greater than 2 in both media as shown in Table I. It is believed that microorganisms can be considered potentially viable for biotechnological use when they have an enzyme index > 2.0 (Stamford et al. 1998), generating relevance for *Penicillium* sp.1 (R26). It is assumed that the higher the enzyme index, the greater the enzyme production and, consequently, the greater degradation of target compounds. In this sense, the information found may be in agreement with the data presented by Abdullah et al. (2020), which describes indigenous fungi dominance on soil contaminated by crude oil belonging to *Penicillium* and *Aspergillus* genus and indicating *Penicillium* sp. as more efficient in breaking polycyclic aromatic hydrocarbons, reaching levels between 25% to 32% compared to 13% to 19% when mineralized by *Aspergillus* sp.

When observing the colony diameter values (Table I), *Aspergillus* sp. and *Byssochlamys* sp. showed greater growth, reaching 9.0 and 4.8, 7.8 and 5.9, respectively. Thus, it is noted that colonies with a higher enzyme index were not necessarily the same strains that showed greater radial growth, that is, the microorganism development is not directly proportional to the target enzyme production.

As shown in Figure 2, we can see the experimental design results for all the five strains studied. Time and absence or presence of oil in relation to IE significance were the main effects factors, with factor 2 being more significant, however the interaction factor between them was not statistically significant. Those factors' effect was positive, that is, the longer the time and the presence of oil, the better IE results in *Aspergillus* sp. and *Penicillium* sp. 1 (fig. 2.a and 2.b). As for *Penicillium* sp. 2, only the oil presence

showed significance (p value = 0.004), with a positive effect of 2.73 yield. On the other hand, in *Penicillium* sp. 1 (R26) and *Byssochlamys* sp. strains, all factors and interaction positively influenced the enzyme index. In this sense, it is worth increasing oil concentration to obtain better EI results. Similar results were found by Chen et al. (2019), where enzymatic assays revealed that the activity of manganese peroxidase in *P. simplicissimum*, was significantly induced in cultures supplemented with the contaminant when compared to the control (without contaminant). There are few reports in the literature regarding lignolytic enzymes production from petroleum products or even from crude oil in solid media. However, solid state fermentation (SSF) has been considered as a promising alternative for obtaining enzymes, as it offers a simpler fermentation medium, can provide higher productivity, allows greater control of microbial contamination, uses less water and consequently produces less waste liquid (Atagana et al. 2006). In addition, SSF has great potential for capital reduction in further processing and production, especially when filamentous fungi and industrial waste are used and, therefore, assists in the reuse and recovery of this waste, mitigating environmental impacts arising from improper disposal or accidents (Pires et al. 2020). Therefore, enzyme production in solid fermentation, with desirable characteristics for bioremediation, would help to develop bioprocess technology to obtain bioproducts useful in reducing pollutants toxicity.

For Pz, all effects were statistically significant, including factor 1 interaction with factor 2. However, the effect was negative, as expected, suggesting that the longer the time and the presence of oil, the lower the Pz value in *Aspergillus* sp., *Penicillium* sp. 1, *Penicillium* sp. 2, *Penicillium* sp. 1 (R26), and *Byssochlamys*

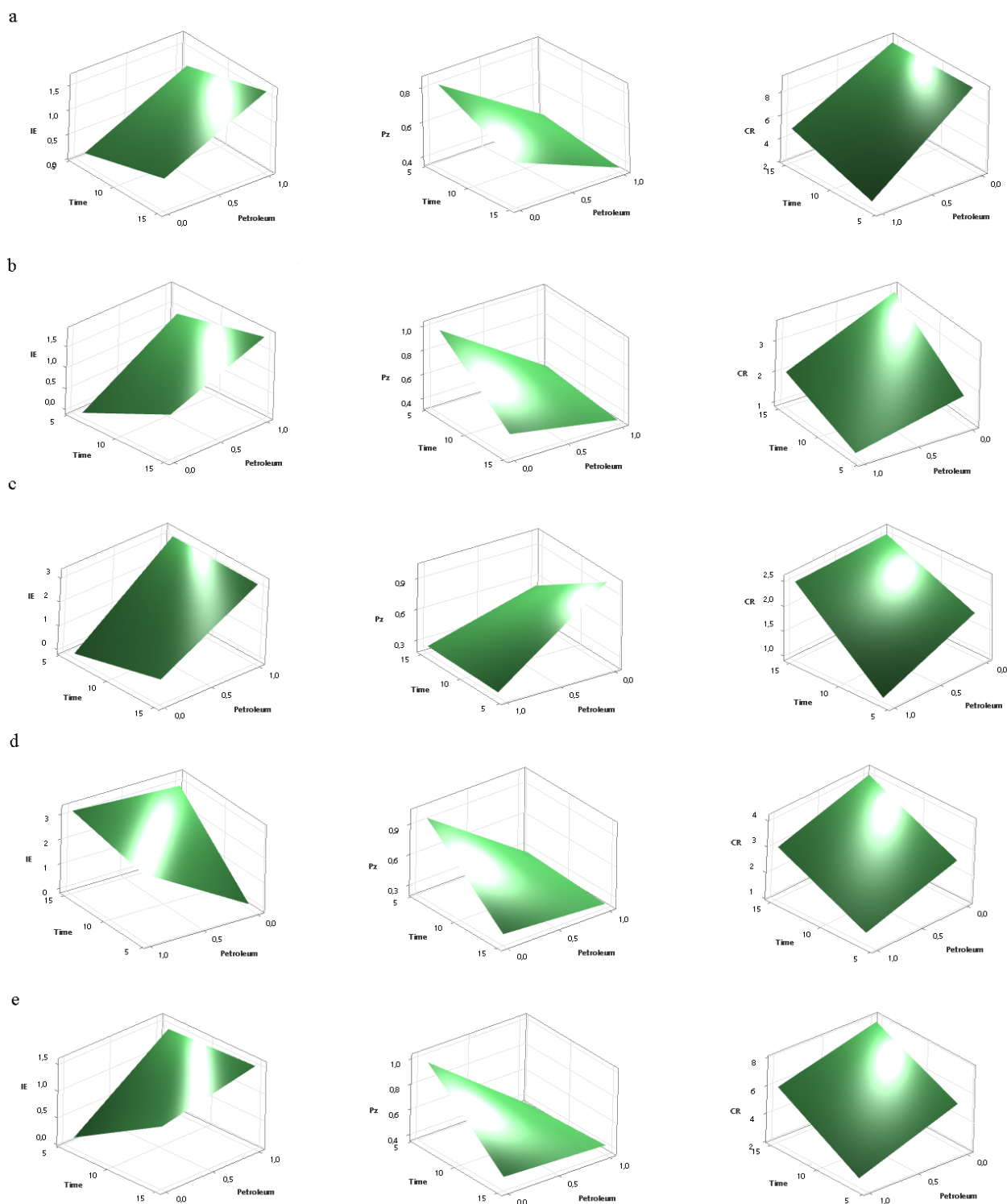


Figure 2. Three-dimensional response surface plot for MnP production using *Aspergillus sp.* (a), *Penicilium sp. 1* (b), *Penicilium sp. 2* (c), *Penicilium sp. 1R26* (d) and *Byssosclamyces sp.* (e) in solid fermentation.

sp., consequently the enzyme activity will be inclined towards strongly positive. The Pz data corroborate the IE findings, showing an inversely proportional relationship. In this sense, the increase in IE and the reduction in Pz demonstrate better results for the evaluation of the studied strains. The Pz and IE indexes were not previously used to evaluate the production of MnP in solid culture medium, however it represents a simple and efficient strategy to indicate studied species potential. Therefore, this method makes it possible to increase the range of screening options for fungi that produce ligninolytic enzymes for laboratories which does not have robust equipment.

Penicillium sp. 2 growth showed significance ($p = 0.005$) for factor 1, that is, it was influenced only by time with a positive effect. Apparently, both media did not provide a significant difference for the strain development. There was no change in growth, but there was an increase in IE when in BH medium. Factors effects and their combination for radial growth (CR) in *Aspergillus* sp. showed a p-value lower than 0.05, and therefore, demonstrated significance in this strain development. On the other hand, the effect was negative in oil presence, that is, the strain grew more in the SDA culture medium than in BH medium, but IE values were favored. The same occurred for *Penicillium* sp. 1., *Penicillium* sp. 1 (R26) and *Byssosclamyces* sp., however the factors interaction was not significant. Thus, even though these strains' growth has declined, the contaminant interference allowed to obtain better EI and Pz values, emphasizing the evaluating importance of agreement between the variables studied. This result was also verified by Heinz et al. (2015), which highlights the need to evaluate the strain as a good enzyme producer, taking into consideration both microorganism growth in culture medium, as well as the enzyme index data.

A possible alternative to improve production in less time would be to increase the tested fungal biomass or change the contaminant concentration, evaluating degradation. Reports on fungi production in co-culture obtaining favored biomass over monoculture and the interaction between species reflects a greater tolerance to contaminant inhibitory effects (Hechmi et al. 2016), leading us to believe that these enzymes production limitations can be overcome by fermentation with multiple colonies. Such characteristics can be observed by other authors, such as Benoit-Gelber et al. (2017) where co-cultivation of *A. niger* with *A. oryzae* resulted in a more complex enzyme profile, Hu et al. (2011) obtained higher enzyme production from mixed cultivation of *A. niger* with *P. chrysosporium*, Zhao et al. (2019) reported the co-cultivation of *P. oxalicum* and *T. reesei* under solid state fermentation to produce enzymes of biotechnological interest and obtained yields up to 4 times higher and Hechmi et al. (2016) obtained increased production with *B. nivea* in cultivation with *S. brumptii*. In addition, microorganisms production in mixed cultures becomes a promising alternative for identifying new enzymes or compounds (Wösten 2019). Moreover, manganese peroxidase multiple production by the five strains can be encouraged to maximize the yield of the process.

CONCLUSIONS

Through the results obtained it was possible to infer that the studied fungi exhibited ligninolytic enzymatic activity, showing a capacity for the production of the enzyme manganese peroxidase with strongly positive enzyme activity values, with values of $IE > 2$ for the genus *Penicillium*, thus providing a scope for their future application for the purpose of bioremediation

of environments impacted by oil and / or its derivatives. The factorial design indicated that the enzyme index was increased in solid state fermentation when in BH medium plus oil, indicating the potential of this contaminant in the production of the MnP enzyme. Taking into account that fungi of marine origin can be an important resource for use in biotechnological processes and knowing the abilities of the strains studied to develop in the presence of petroleum hydrocarbons, the results of this study stimulate further investigations on the production of ligninolytic and other enzymes by *Aspergillus* sp., *Penicillium* sp. and *Byssoschlamys* sp. in addition to concentrating investigations on the characterization of these enzymes and on the optimization of the high biomass production process, in order to develop a bioproduct with multiple degradation functions.

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SUPPLEMENTARY MATERIAL

Figure S1. Phylogenetic tree based on ITS analyzes showing closest relatives of isolates of fungi derived from the sea (model of neighbors' union and bootstrap of 1000 replicates).

Table S1. Molecular identification of filamentous fungi from mangrove sediment in the bay of Todos os Santos, Brazil

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