Organic wastes for biostimulation of *Agaricus bisporus* and *Pleurotus ostreatus*

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Abstract

Ligninolytic fungi have been tested to degrade organic pollutants with excellent results because of their extracellular activity. Some of these fungi are cultivated for human consumption with the consequent co-generation of massive amounts of spent mushroom substrate. The main objective of this work was to obtain of an organic material from agricultural or industrial wastes to achieve the revitalization of the mycelia remnant in spent mushroom substrates from the edible fungi *Agaricus bisporus* and *Pleurotus ostreatus.* To reach this goal a study of the impact of the different organic materials in the mushroom behaviour has been made with and without a sterilization treatment to determine the evolution of the enzymatic activity in absence and in presence of microbial competence. In addition to the enzymatic analysis of laccase, manganese peroxidase (MnP) and versatile peroxidase (VP), Cu and Mn were determined to find out if they had an impact in the enzymatic activities. The four selected amendments were goat manure compost (GC), spent mushroom compost of *P.ostreatus* and *A. bisporus* (SM), wheat straw (WS) and deinking sludge from paper mill (PW). The results showed that the amendment factor had more influence in the enzymatic activity than the fungi specie factor. The WS is the amendment with most potential in biostimulation because of its high enzymatic activity stimulation, which was also the most persistent over time.

Keywords: bioremediation, laccase, agricultural wastes, ligninolytic fungi

1. Introduction

Ligninolytic fungi have been used to degrade organic pollutants such as synthetic dyers, polycyclic aromatic hydrocarbons, heavy oils or pharmaceutical compounds including antibiotics [1]. This kind of fungi has developed a unique extracellular enzymatic system capable of degrading lignin by producing free radicals. This mechanism allows the enzymes to be catalytically active in a great variety of organic substrates, which provide the ligninolytic fungi a potential use in bioremediation.

Some of these fungi, like those of genus *Agaricus* and *Pleurotus*, are cultivated for human consumption with the consequent co-generation of massive amounts of spent mushroom substrate [2]. Previous works have proved the usefulness of spent mushroom substrate to degrade organic pollutants and the role of the active fungal mycelium in this process [3]. One important step, with practical consequences for their use in bioremediation purposes consists in the maintenance over time of their extracellular enzymatic activities, in order to metabolize pollutants in both, solid matrices as soil or organic wastes, and liquid effluents. One option to evaluate the fungi development consists in the determination of the ergosterol concentration, although each fungal specie has its own ergosterol levels considering that it is an intrinsic characteristic of each fungus specie [4].

Some characteristics of the organic materials define the different behaviour of growth, development and expression of enzymatic activities. The C/N relationship has influence in the ligninolytic enzymes production [5], as well as humidity and pH (4.5 optimum pH for fungi growth). Basic pH inhibits mushroom growth due to microbial competition and not by the nutrient limitation [6]. Some fungi have an increment in their enzymes production in N deficient conditions [7]. White rote fungi use N with great efficiency, they even recycle aged mycelia. In poor N conditions, they allocate N to extracellular enzymes production apart from essential cellular components [8]. According to [9], *P. ostreatus* decreases its enzymatic activity with inorganic N input but in some forms of organic N (especially low molecular weight fractions) they experiment an enzymatic activity increase due to the higher biomass production [10]. Also, Meidute et al 2008, [11] concludes that N addition favours mycelia growth.

The goal of this work consisted in the obtainment of an organic material from agricultural or industrial wastes to achieve the revitalization of the mycelium remnant in spent mushroom substrates from *A. bisporus* and *P. ostreatus.*

To reach this objective it is necessary that the fungi grow and survive while producing ligninolytic enzymes in bioremediation conditions, including microbial competition. Fungal extracellular peroxydases need to be transported through the soils to contact with pollutants [12].

During this work a study of the impact of ~~the~~ different organic materials in fungi behaviour has been made with and without a sterilization treatment to determine the evolution of the enzymatic activity in absence and in presence of microbial competence. In addition to the enzymatic analysis, Cu and Mn were determined to find out if they had an impact in the enzymatic activities. The extracellular ligninolytic enzymes studied were laccase, manganese peroxidase (MnP) and versatile peroxidase (VP).

All the organic material and spent-mushroom substrates were characterized. This analysis was necessary for the evaluation of the potential to generate, immobilize and degrade pollutants of organic residues, and their influence in the fungi growth and enzymatic activity.

2. Materials and methods

2.1. Organic amendments and mushroom spent substrates

The organic amendments were obtained from different companies as by-products of their activities. The four amendments were compost from goat manure (Suerte Ampanera, Madrid) (GC), re-composted spent mushroom compost of *P. ostreatus* and *A. bisporus* (Compost Villacasa, Cuenca) (SM), wheat straw (Compost Villacasa, Cuenca) (WS) and deinking sludge from paper mill (Hollmen Paper, Madrid) (PW). All samples were air-dried, the wheat straw was sliced to 5-30 mm length fragments and the deinking sludge was sieved through 2 mm mesh.

2.2. Evaluation of the impact of several organic wastes in the revitalization of mycelium of spent mushroom substrates.

A factorial experimental design was carried out with two fungal species (*A. bisporus* and *P. ostreatus*), four organic amendments (GC, SM, WS, PW) and two treatments sterilized (E) in autoclave 120ºC and 1.2 atm pressure for 30 minutes and not sterilized (X). Different fungi, materials and treatments were placed in 1 L glass flasks with 20 g of fresh spent mushroom substrate and 20 g of every material tested, with a 75% moisture regarding dry weight, with four replicates for each sample. Every flask was aired every 48 hours for 30 minutes, the (E) treatments in a laminar flow hood to avoid contamination with exogenous microorganisms.

Aliquots of each material and treatment were sampled after 14 and 28 days of incubation in the dark at room temperature. Ergosterol, as index of fungi growth, and the ligninolytic activities laccase (Lac), manganese peroxidase (MnP) and versatil peroxidase (VP) were determined.

Carbon and Nitrogen were determined with a LECO CHNS-932 Elemental Chemical Analyzer, pH and Electrical conductivity (EC), and water-soluble organic carbon (WSOC) were determined in the aqueous extract 1:10 (w/v) according to [13, 14], Cu and Mn were determined in triplicate according to [13].

The Solid-State nuclear magnetic resonance, ^{13}C-CP-MAS NMR spectra of the organic amendments was performed using a Bruker AV-400-WB unit at 300 K. Samples were packed in a 4 mm diameter zirconium rotor with Kel-F cap, with a rotor spin rate set at 14 kHz. For each sample of about 100 mg, 28.500 scans were accumulated with a relaxation delay of 2 s and 5 ms of contact time. In the ^{13}C-CP-MAS NMR spectra the chemical displacement (δ) was divided in the main resonance regions according to [15].

2.3. Ligninolytic enzymatic activities

The determination of the ligninolytic enzymes (laccase, manganese peroxidase (MnP) and versatile peroxidase (VP) were made in the enzymatic extract according to [1]. To obtain this extract, 30 mL of the buffer Tris-HCl 0.1 M pH 7.5 was added to 1.5 g of each sample. The mixture was washed under agitation (160 rpm) in a water-ice (4 ^{o}C) bath for 60 minutes. Afterwards, the samples were centrifuged for 10 minutes at 5000 rpm. The supernatant was separated for the enzymatic analysis.

Laccase activity was determined by the oxidation of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) to $ABTS^{+*}$ by laccase [16]. $ABTS^{+*}$ is green, which allows its spectrometric measurement at 420 nm. 880 µL of the potassium acetate buffer pH 4.5, 110 µL of ABTS 1.8 M and 10 µL of sample were mixed in the cuvette and the oxidation of ABTS was monitored during 10 min 30 ^{o}C. To prepare the blank the 10 µL of sample were substituted by buffer [17].

MnP activity was determined by the oxidation of Mn^{2+} to Mn^{3+} by MnP. Mn^{3+} forms a malonate-Mn^{3+} complex with an absorbance maximum at 270 nm. 950 µL of malonic sodium malonate buffer pH 4.5, 10 µL of $MnSO_4$ 1 mM, 10 µL H_2O_2 10 mM and 30 µL of sample. In the blank the 30 µL of sample were substituted by buffer. The process of taking measures was the same as in the determination of laccase activity with the difference of an increment of incubation time to 30 minutes [17, 18].

VP was determined by the oxidation of ABTS in presence of H_2O_2, the process of measurement was the same as the determination of laccase but changing the reagents to 870 µL of potassium acetate buffer pH 4.5, 110 µL of ABTS 0.2 mM, 10 µL of H_2O_2 0.1 mM and 10 µL of sample [17].

2.4. Ergosterol determination

Ergosterol content was determined after 14 days of incubation following the method developed by [19]. 0.5 g of sample (previously grinded to particles under 0.5 mm) were weight and sonicated at 70 ^{o}C with 3 mL of a solution of KOH 10% in methanol for 90 minutes. The extract was filtered (Whatman 42) and the solid residue was washed with 3 mL of methanol. 1 mL of distilled water was added to the filtrate, which was extracted 3 times with 3 mL of hexane. The hexane phase was dried under N_2 flow. Finally, the dry residue was redissolved with 3 mL of methanol to perform analysis by HPCL (Waters 2695 Separation Module, Waters Milford, MA) equipped with a Phenomenex Luna C18 column (250 mm x 4.60 mm; particle size 5 µm; pore size 100 Å) and PDA detector (Waters-PDA 996). The HPLC-PDA conditions applied were: methanol:water (94:5, v:v) as mobile phase, 1.0 mL/min of flow rate and 20 µL of sample injection volume and a 30 minutes of chromatographic analysis. The elution profile was monitored at 282 nm. Ergosterol was identified by its UV spectrum (200-400 nm).

2.5 Statistical analysis

The results were statistically analyzed using SPSS v. 23. Before any statistical analysis was made, the non-equal variables by the Levene's test were transformed by SPSS and all data was determined to be in a normal distribution according to the Shapiro-Wilk (N<50) criteria. The means comparison was made with two-way ANOVA while the influence of the Mn^{2+} and Cu^{2+} cofactors was studied by ANCOVA. The Pearson's correlation coefficient was also calculated to appraise the linear correlation of the several variables. The amendments controls without mushroom were dismissed to the statistical analysis of ligninolytic activities and ergosterol due to its low content, were not quantifiable by the chosen methods.

3. Results and discussion

3.1 Solid state nuclear magnetic resonance (^{13}C CP-MAS NMR)

The chemical shifts of ^{13}C-MAS NMR of the several amendments are represented in the table 1. The chemical shifts were divided in seven regions according to [15]:

Table 1. Chemical shifts of ^{13}C-MAS NMR of the amendments

C bond (δ) ppm	% Area			
	GC (X)	SM (X)	WS (X)	PW (X)
Alkyl (0-50)	25.1	16.8	3.8	23.3
N-alkyl (50-60)	10.9	5.0	3.7	4.0
O-alkyl (60-110)	42.9	59.4	81.1	52.4
Aromatic (110-140)	5.8	7.7	2.4	11.2
O-aryl (140-160)	4.9	6.1	2.9	5.3
Carboxyl (160-190)	9.4	4.9	3.0	2.8
C ketone and amide (190-220)	1.0	0.1	1.1	0.8

The peaks at 72 ppm belongs to the carbon C_1 and the peak at 105 ppm to the carbon C_2, C_3 and C_5 of the cellulose and hemicellulose [20]. These peaks are more intensive in the WS and PW amendments, which means that they are richer in these compounds. The peaks at 55 and 153 ppm belong to the methoxy and phenyl groups of lignin [20]. The WS is the amendment with more lignin content. In the

region between 160 y 190 ppm, the peak at 173 ppm is associated to de carboxylic C, this peak raises with the compost maturation rate, it shows that GC is an amendment with more maturation rate than the SM [21].

It can be observed in the table 1 that all amendments have its mayor region in the O-alkyl fraction that mainly belong to cellulose and hemicellulose, the WS being the one with more percentage of this fraction. The second majority fraction is related to the alkyl structures, probably associated to aliphatic structures and organic acids. For the PW amendment this region is lower.

3.2 Sterilization effect in the substrates

The levels of C and N and their ratio indicated that highest C/N of wheat straw (WS) in comparison with the other agricultural wastes (table 2). The results of the pH, CE and WSOC determinations are displayed for materials sterilized (E) and no sterilized (X) in the table 2.

Table 2. pH, Electrical Conductivity (EC), water soluble organic carbon (WSOC) of wastes sterilized (E) and no sterilized (X). Carbon, nitrogen, C/N ratio and pseudo total Cu and Mn concentrations. GC= Goat manure compost; SM= Spent mushroom compost;.WS= wheat Straw; PW= paper mill waste. Different letters denote significant differences between treaments ($p<0.05$)

		GC	SM	WS	PW
C (%)	**X**	14.2	23.7	43.8	23.0
N (%)	**X**	1.5	2.5	0.4	0.4
C/N	**X**	9.5	9.5	109.5	57.5
	E	7.58	7.17	5.55 b	8.01
pH	**X**	7.65	7.20	5.82 a	7.76
	E	3.24	7.47 a	3.53 a	0.40
EC (dS/m)	**X**	3.38	6.99 b	2.84 b	0.44
	E	584.6 a	580.5 a	2347.3 a	304.4
WSOC (mg/kg)	**X**	236.7 b	350.4 b	1831.4 b	282.2
Cu (mg/Kg)	**X**	10.90	50.29	1.15	195.90
Mn (mg/Kg)	**X**	428.9	400.3	47.86	63.78

pH values indicated the more acidic conditions of WS in comparison with other wastes tested. The sterilization by autoclave affects to several chemical properties such as pH in WS, EC in SM and WS and WSOC in GC, SM and WS. Sterilization does not affect to the pulp mill waste (PW). The sterilization produced a significant acidification of WS. The pH decrease could be associated to the most volatile compounds (organic acids of short and long chains). These acids can be produced due to oligosaccharides and amino acids degradation, and because of the decomposition of the phenols originated in the lignin solubilisation [22, 23]. In addition, this reduction of pH can be related to the ester-type bonds breakdown in hemicellulose and lignin molecules [24].

EC measurements were statistically significant in the WS and SM substrates, increasing a 20 % and a 6.5 % respectively. The sterilization induces the humus and residual biomass bonds rupture; accordingly, several ions were released to the solution which increases the EC. According to [25] sterilised soils have a higher EC than non-sterilised soils.

Water soluble organic carbon concentration (WSOC) increased significantly in all materials apart from PW. The raise of WSOC fraction in both compost comes from the organic acids released from the humic fraction and the organic matter hydrolysis. The cellulose from the PW and WS residues suffers a hydrolysis process where C-C bonds broke and carbohydrates depolymerise [26] which increases the WSOC fraction.

3.3. Sterilization and amendments effects in ergosterol content

According to the table 3, the concentration of ergosterol after 14 days of incubation in amendments where *A. bisporus* was inoculated showed no significant differences between the sterilized and no sterilized treatments. *A. bisporus* in PW showed less growth than in control while in GC the fungi had the opposite

behaviour, it showed more growth than in the control. In SM and WS substrates there were not significant differences with respect to the control.

Table 2. Ergosterol concentration (mg kg^{-1}) after 14 days of incubation in *A. bisporus* and *P. ostreatus* GC=Goat Compost. SM=Spent-mushroom compost. WS=wheat straw. PW=paper mill waste. X=no sterilized treatment. E=sterilized treatment. The letters in the same column show the significant differences between the amendments according to the Duncan test ($p<0.05$).

	A. bisporus	***P. ostreatus***
GC (E)	21.0 ± 0.4^{c}	61 ± 8^{c}
GC (X)	21 ± 1^{c}	60 ± 5^{c}
SM (E)	11.5 ± 0.9^{b}	34 ± 4^{ab}
SM (X)	19 ± 1^{b}	38 ± 11^{ab}
WS (E)	18 ± 2^{b}	41 ± 3^{b}
WS (X)	13.7 ± 0.5^{b}	35 ± 5^{b}
PW (E)	6.5 ± 0.8^{a}	19 ± 2^{a}
PW (X)	9.8 ± 0.8^{a}	24 ± 3^{a}
Control	13 ± 2^{b}	87 ± 9^{d}

Ergosterol concentrations obtained in amendments with *P.ostreatus* were higher than those with *A. bisporus* indicating the different synthesis of this molecule for each fungal specie and the impossibility to perform a comparative quantification between them. Even so the trends obtained for *P. ostreatus* were the same than in *A.bisporus* except for the control, which shows significantly higher concentrations than all amendments tested. In the same way than *A. bisporus*, there were not significant differences between E and X treatments.

3.4. Effects of amendments and fungal species in enzymatic activity

The fig. 1 compares the laccase enzymatic activity evolution during two sampling periods (14 and 28 days). Activities were determined for each specie and each amendment depending on the sterilization treatment (fig. 1 a for sterilized amendments and fig. 1 b for no sterilized). It can be noticed that the PW had a considerable increase of laccase activity at t_{14} but it decreased drastically at t_{28}. This rise can be attributed to the high Cu^{2+} concentration in the amendment (table 2). *A.bisporus* control presented a high laccase activity but also decayed at t_{28}. *P.ostreatus* control had less enzymatic activity than the amendments at t_{14} and t_{28} therefore amendments were effective to stimulate the production of laccase by *P. ostreatus*. Similar result was reported in polluted soil amended with biochar and inoculated with *P. ostreatus* using wheat straw as fungal carrier [1]. However, the positive effect of organic amendments was not effective at lasting time. In contrast, laccase activity of *A. bisporus* was constant during the assay in presence of WS. Compost amendments (GC and SM) were not able to maintain laccase activity and PW produced a strong decrease of laccase activity between 14 and 28 days. Therefore, WS was the most effective amendment maintaining the laccase activity.

The determination of laccase activity in non-sterile conditions (Fig. 1 b) simulating conditions with microbial competence and evaluated the possibility of growing the fungi in those conditions before applying the amendments in soils with the subsequent energy saving. In general, under non-sterile conditions laccase activities were slightly lower than those obtained in sterilized conditions, so microbiological competence could avoid the expression of this enzyme in some extension. The activation of this enzyme in the amendment PW, explained with the higher Cu levels of this organic waste has not place in the same extension without sterilization. If the fig. 1 a and 1 b were compared, it can be observed than at t_{14} the sterilized treatments had higher laccase activity but the differences between t_{14} and t_{28} were more pronounced. The no sterilized *P.ostreatus* amendments showed slight variation of enzymatic activity over time. Once again, WS and PW were the most effective amendment to preserve the laccase activity of *A. bisporus* and *P. ostreatus*. Both fungi showed significant laccase activity after 28 days of

incubation, specially *P. ostreatus* that presented similar enzymatic activity at 14 and 28 days in WS and PW.

[27][27]Data obtained showed that laccase activity was not related to ergosterol, specially in amendment PW, where high laccase activity was found but not fungi growth according to ergosterol. Cu concentration could have some incidence in the expression of laccase in PW but it was the only scenario were some incidence of Cu presence could condition this enzymatic activity.

The fig. 2 (a and b) shows the evolution over time of the manganese peroxidase (MnP) activity in the different amendments sterilized and not sterilized. *A.bisporus* control had the maximum activity at t_{14} but decreased drastically at t_{28}. In contrast, *P.ostreatus* control increased the MnP over time. The P-WS (fig. 2a) got more activity than A-WS (E) and moreover maintained the activity at t_{28}. Both compost (GC and SM) presented levels below the rest of amendments at t_{14} and t_{28}. Therefore, WS was the most promising amendment for *P. ostreatus*.

In *A.bisporus* the decrease of MnP activity was the general trend over time except with GC that enhance MnP at t_{28}. *P.ostreatus* showed activity at t_{14} and decreased at t_{28} in all the treatments apart from the control where it continued growing.

The general behaviour in the no sterilized amendments (fig. 2b) was similar to the sterilized treatments but with lower values, the same that happened with laccase activity. *P.ostreatus* with sterilized straw trends to maintain its enzymatic activity over time, both laccase and MnP. *P.ostreatus* showed a stable MnP activity over time in straw in both treatments (sterilised and no sterilised). *A.bisporus* presented the same behaviour with sterilized and no sterilized WS.

The amendments did not affect to the enzymatic activity versatile peroxidase at t_{28} with the exception of *A.bisporus* with SM and WS, that increased their activity at t_{14} but decreased again at t_{28}. The difference of VP with respect to the other activities was that the tendency of VP at t_{28} was not a drastically decrease.

Table 4. Versatil peroxidase activities (U/g) after 14 and 28 days of incubation in *A. bisporus* (A) and *P. ostreatus* (P). GC=Goat Compost. SM=Spent-mushroom compost. WS=wheat straw. PW=paper mill waste. X=no sterilized treatment. E= sterilized treatment.

VP	Sterilized (E)		No Sterilized (X)	
	t_{14}	t_{28}	t_{14}	t_{28}
A. bisporus				
Control	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
A-GC	0.5 ± 0.1	0.05 ± 0.05	ND	0.2 ± 0.1
A-SM	0.02 ± 0.1	ND	0.2 ± 0.1	0.10 ± 0.04
A-WS	0.3 ± 0.1	ND	0.3 ± 0.1	0.02 ± 0.02
A-PW	0.3 ± 0.2	0.12 ± 0.2	0.06 ± 0.04	ND
P. ostreatus				
Control	ND	0.3 ± 0.2	ND	0.3 ± 0.2
P-GC	ND	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.3
P-SM	0.03 ± 0.02	0.5 ± 0.2	0.07 ± 0.03	0.3 ± 0.1
P-WS	6 ± 1	5.2 ± 0.3	2.0 ± 0.5	2.0 ± 0.8
P-PW	2.6 ± 0.9	0.5 ± 0.2	0.6 ± 0.2	0.4 ± 0.2

The WS and PW were the only residues which stimulated versatile peroxidase activity in *P.ostreatus* after 14 days of incubation. In wheat straw the activity was maintained in time t_{28}, and significantly reduced during the same period in PW. These amendments are the ones that had less Mn concentration (table 2) meaning that with less Mn content more peroxidase activity independently of Mn (VP) is produced.

The WS was the amendment with more VP activity and *P.ostreatus* showed more peroxidase activity than *A.bisporus.*

3.5. Biostimulation of A. bisporus and P. ostreatus by organic wastes applied to the spent-mushroom substrate

After results shown, the criteria to select the most adequate amendment to express and maintain the extracellular enzymatic activities were: High enzymatic activity and/or conservation over time, less microbial competition and fungi growth described as ergosterol concentration. Cu could be a factor to stimulate laccase, according to obtained results in PW at t_{14} but it was not a decisive factor, so it decays drastically at t_{28}. The decay that suffers at t_{28} could be related to the high pH of PW (8.01 in E and 7.76 in X, table 2) that was the highest of all the organic wastes assayed.

Both compost assayed, GC and SM were the amendments with less enzymatic activity. Although they had a high Mn^{2+} concentration, their MnP activity did not reach adequate enzyme values for bioremediation. [10] observed that the MnP gene transcription is regulated by a mechanism that combines repression and activation in a nested way that means that the Mn^{2+} produces different effects depending on the state of the transcription in the moment of addition and it can inhibit the MnP expression. On the one hand the compost pH (7.10-7.65) favoured more the bacterial growth than the fungal growth and on the other hand the C/N relationship was low to the enzymatic stimulation [9].

WS had an adequate pH to the mycelium development and a C/N relationship that could be optimal to stimulate the ligninolytic enzymes production [8]. Laccase and MnP activities maintained their levels over time and with a high activity index. The peroxidase activity with the WS amendment almost triplicate the activity of the other treatments. The advantage of this enzyme for bioremediation is its ionization potential, much higher than the potential of laccase [31] that is the main enzyme in the other wastes assayed and has its degrading limitation in the low redox potential [32]. Because of these reasons, WS could be the best amendment to enrich the spent-mushroom substrate.

Both control treatments presented high MnP and laccase activity in *A.bisporus* at t_{14} but at t_{28} this activity decreased drastically (with the exception of MnP in *P.ostreatus* that increased).

According to the species, *Agaricus* presented higher values in some cases for enzymatic activities laccase and MnP. However, *P.ostreatus* persisted over time and its peroxidase activity was higher.

In addition. *P.ostreatus* was more competitive and vigorous [12] if we consider the higher ergosterol content which increased in comparison with the control.

The values of no sterile treatments (X) were lower than the sterile treatment (E). This fact suggested that the amendments should be incubated for at least 14 days in this condition before their application to soils. If the circumstances did not allow the incubation the direct application to soils would likely have success.

4. Conclusions

The sterilization of amendments did not have influence in fungi growth of *P.ostreatus* specie but it had a little impact on *A.bisporus*.

Regardless the specie, PW was the amendment which ~~caused~~ more enhanced stimulation of laccase activity due to its high Cu content, and its concentration could affect more than the specie factor itself in the laccase activity. Versatil Peroxidase activity was the most stimulated of all activities. The peroxidase activity without dependence of Mn (VP) was more stimulated when the Mn content was lower in the amendment.

Although *P.ostreatus* was competitive and adequate to bioremediation the amendment factor had also influence in the enzymatic activity. According to the results presented, WS was the best amendment for bioremediation purposes because of its capability of enzymatic stimulation which was also the most persistent over time. This durability gave to WS an advantage over the rest of the organic wastes which can be applied in environmental remediation.

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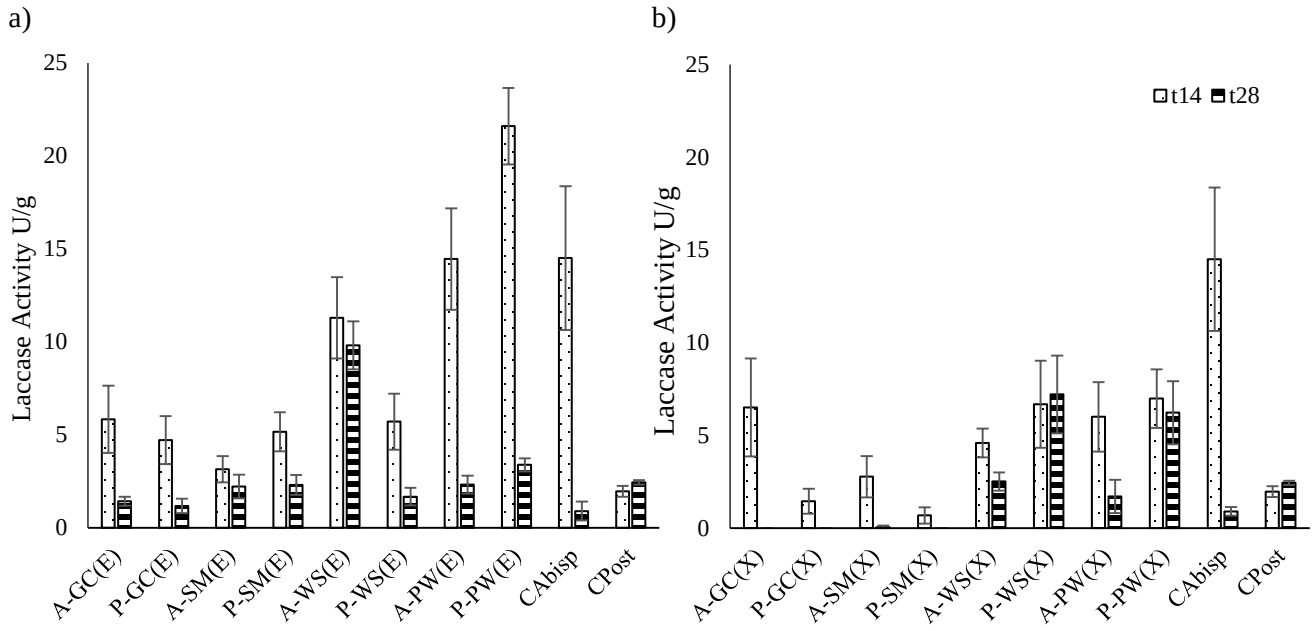

Fig. 1. Laccase activities (U/g) after 14 and 28 days of incubation in *A. bisporus* (A) and *P. ostreatus* (P) a) Sterilized and b) no sterilized. GC=Goat Compost. SM=Spent-mushroom compost. WS=wheat straw. PW=paper mill waste. X=no sterilized treatment. E= sterilized treatment.

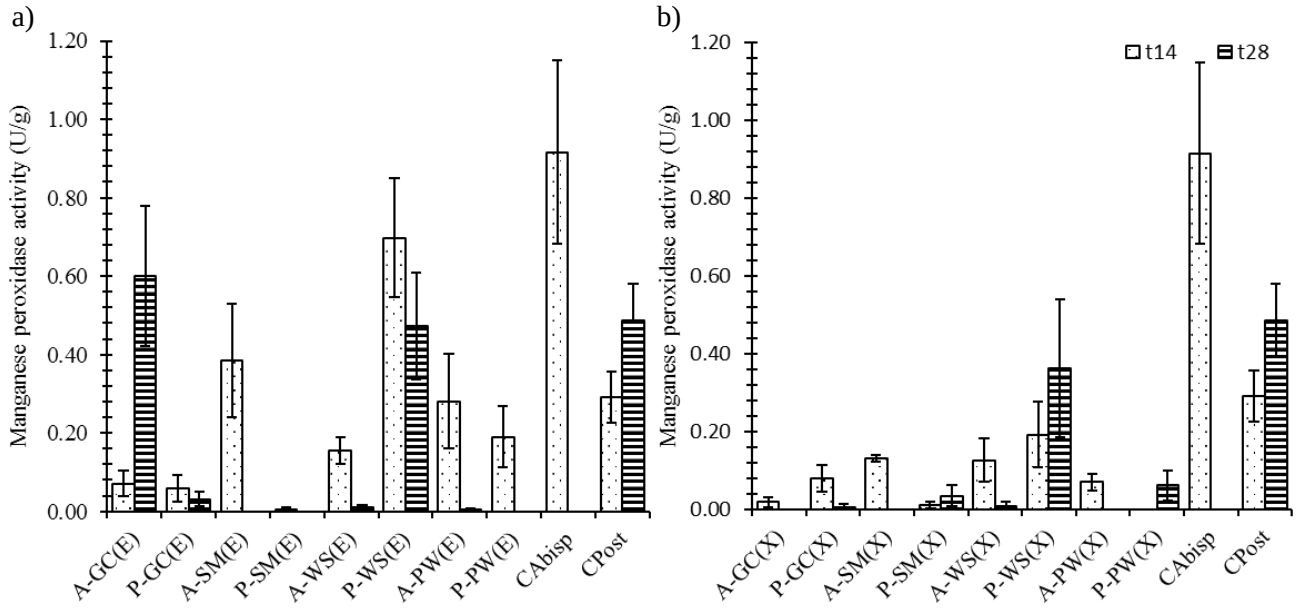

Fig. 2. Manganese peroxidase activities (U/g) after 14 and 28 days of incubation in *A. bisporus* (A) and *P.*

ostreatus (P) a) sterilized; b) No sterilized. GC=Goat Compost. SM=Spent-mushroom compost. WS=wheat straw. PW=paper mill waste. X=no sterilized treatment. E= sterilized treatment.