Effects of biological pre-treatment of lignocellulosic waste with white-rot fungi on the stimulation of ^{14}C-phenanthrene catabolism in soils.

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Abstract

The enhancement of phenanthrene catabolism in soils amended with lignocellulosic waste material (spent brewery grains) was investigated. The soils were pre-treated with five white-rot fungi (*Phanerochaete chrysosporium*, *Trametes versicolor*, *Irpex lateus*, *Pleurotus ostreatus,* and *Bjerkandera adusta*). The changes in the kinetics of ^{14}C-phenanthrene mineralisation (lag phases, the fastest rates and the overall extents) were measured in the inoculated, PAH-amended soils over time (1–100 d). Changes in the ligninolytic (laccase, lignin peroxidase and manganese peroxidase) and non-ligninolytic (β-glucosidase and phosphatase) enzymatic activities were also assessed. Overall results revealed that the amendment of fungal pre-treated SBG influenced the kinetics of mineralisation of ^{14}C-phenanthrene as well as the enzymatic activities in soils. Soil inoculated with fungal pre-treated SBG caused reductions in lag phases as well as higher rates and extents of ^{14}C-phenanthrene mineralisation in the following trend *T. versicolor* > *B. adusta* > *P. chrysosporium* = *P. ostreatus* > *I. lateus*. Furthermore, the extents of mineralisation generally reduced as levels of ligninolytic enzyme decreased, while the non-ligninolytic enzymes increased with soil-PAH contact time in all amendment conditions. These findings provided an insight on the potential of biological pre-treatment of waste materials for enhanced carbon, energy and nutrients on the bioactivities and biodegradation of organic pollutants which may be applicable during *in situ* remediations of contaminated soil.

Keywords: Lignocellulose, white-rot fungi, phenanthrene, biological pre-treatment, soil, pre-treated SBG

1. Introduction

Hydrophobic organic contaminants (HOCs), such as the polycyclic aromatic hydrocarbons (PAHs), have been widely studied due to their persistence in the environment and the potential risks they pose to human and environmental health (Wilson and Jones, 1993; Idowu et al., 2019). In addition, factors such as contaminant mobility (controlled by their hydrophobicity, lipophilicity, and water solubility), and bioavailability/bioaccessibility may also contribute to their persistence and susceptibility to biodegradation in soil (Semple et al., 2007; Riding et al., 2013). Numerous studies have shown the efficacy of biodegradation in the removal of HOCs from contaminated soils (e.g. Zhang et al., 2006; Peng et al., 2008; Ghosal et al., 2016); this typically depends on the biological and enzymatic activities of inherent microbiota (Pozdnyakova et al., 2012; Ghosal et al., 2016; Ibeto et al., 2020). The metabolic function of microflora and associated enzymes in nutrient-depleted soils accompanied with low contaminant availability can be reduced or remain inactive, resulting in the persistence of the organic contaminant and further affecting the soil ecosystem's health (Breedveld and Sparrevik, 2000; Al-Hawash et al., 2018). Therefore, to stimulate soil microbial and enzymatic activities, including soil fertility and health, the use of organic amendment using lignocellulosic waste materials, is considered a potential approach for bioremediation and nutrient management strategy (soil organic matter and fertility) for PAH contaminated soils.

Lignocellulosic waste materials, especially those generated from the agro-industrial processes, such as rice straw, sugarcane bagasse, corn cobs, spent brewery grains are potential sources of organic nutrients for microbial growth and metabolism in PAH contaminated soils (Brändli et al., 2005; Ren et al., 2018; Omoni et al., 2020a). Soil amended with lignocellulosic materials can provide organic carbon, nitrogen and phosphorus to the soil biota (Larney and Angers, 2012; Chojnacka et al., 2020). These materials can also be colonised and used by microorganisms as ecological niches in soil, especially when they are used as microbial-support systems for contaminant removal, thereby protecting the microbes against environmental stresses associated with organic pollution (Sari et al., 2014; Andriani and Tachibana, 2016).

Lignocellulosic biomass is composed of two carbohydrate polymers (cellulose and hemicellulose) and a non-carbohydrate phenolic complex heteropolymer (lignin); the degradability and digestibility of the biomass vary with the structure of the lignin content (Janusz et al., 2017). Several pre-treatment methodologies have been employed to break down the chemical composition and structure of lignocellulosic biomass residues (Baruah et al., 2018). However, biological pre-treatment methods have some advantages over other pre-treatment methods, such as mechanical methods which are costly due to high energy input and the formation of toxic inhibitory products such as acetic, furural and phenolic acids, as well as high solvent cost associated with chemical pre-treatment strategy (Ramarajan and Manohar, 2017). Therefore, the biological pre-treatment processes are more economically viable, superior and eco-friendly compared to the other pre-treatment techniques (Isroi et al., 2011; Wagner et al., 2018). Biological pre-treatment processes represent promising approaches to the removal of lignin from the waste materials, while increasing enzymatic hydrolysis of the hemicellulosic and cellulosic contents to monomeric sugars such as xylose, arabinose, mannose, glucose and galactose, the readily metabolizable carbon source for microbial growth and metabolism. The white, soft and brown fungi and some bacterial species can delignify and degrade hemicellulose from the lignocellulose. Specifically, the white-rot fungi (WRF), the basidiomycetes, have been extensively studied because of their potential for higher delignification of the biomass as well as simultaneous degradation of cellulose and hemicellulose (Wan and Li, 2010; Isroi et al., 2011; Rouches et al., 2016). This is mainly due to their production of extracellular ligninolytic and non-ligninolytic enzymes

(Baldrian, 2006; Abdel-Hamid et al., 2013), making them potentially viable candidates for biological pre-treatment of lignocellulosic wastes.

The extracellular ligninolytic enzymes involved in lignin degradation include the peroxidases (lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase (VP), dye-decolorizing peroxidase (DyP)) and the laccases (Abdel-Hamid et al., 2013), which can effectively metabolize lignin in a variety of different types of lignocellulose biomass (Isroi et al., 2011). Depolymerisation of the lignin molecules by these enzymes results in materials that are more available for microbial attack, and as sources of nutrients to other soil biota (Saritha et al., 2012; Gao, et al., 2010). However, the lignin-degrading enzymes must be active and stable in the organic substrate (lignocellulose) as well as in the contaminated soil (Lang et al., 1998) for effective biodegradation. Other enzymes, such as the extracellular hydrolases (β-glucosidases and phosphatases) are also secreted by WRF and are known as bio-indicators of soil health, function and quality (Adetunji et al., 2017). These enzymes are involved in biogeochemical cycling of carbon (β-glucosidases) and phosphorus (phosphatases) owing to enzymatic break down of organic matter and nutrient mineralisation in soil (Wali et al., 2020). Previous studies have reported the stimulation of these enzymes by lignocellulosic biomass amendment, including the improvement of soil quality, mainly in PAH-contaminated soils (Tejada et al., 2008; Anza et al., 2019). Very few studies have investigated the addition of organic waste in PAH-contaminated soil with enhanced biodegradation using lignocellulosic material-immobilized WRF (Mohammadi and Nasernejad, 2009; Ros et al., 2010; Lukić et al., 2016), but very few investigations have been reported on PAH degradation in contaminated soils after amendment with biologically pre-treated lignocellulosic waste materials, such as spent brewery grains (SBGs).

For this study, it was hypothesised that the addition of fungal pre-treated SBG would (i) enhance the kinetics of mineralisation of ^{14}C-phenanthrene in soil over time; (ii) stimulate the biological activity (microbial and enzymatic) in PAH-amended soil over time; (iii) develop favourable carbon to nitrogen ratio and pH in the soil to support effective mineralisation of ^{14}C-phenanthrene in soils, and (iv) the fungal pre-treatment would release and increase the accessibility of available sugar monomers and other nutrients from the SBG thereby supporting microbial growth and activity. To address these hypotheses, the aim of this study was to investigate the influence of the addition of fungal pre-treated SBG on the mineralisation of ^{14}C-phenanthrene (a model PAH) in soil and the impact on soil physio-chemical properties and biological activity over time. This was achieved by sampling at different time points (1, 25, 50, 75 and 100 d) to assess the kinetics of mineralisation of 14C phenanthrene (lag phases, fastest rates and extents) in the soil incubations. Five different lignin-degrading white-rot fungal inocula were studied: *Irpex lateus*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor* and *Bjerkandera adusta* (Leonowicz et al., 1999; Hatakka and Hammel, 2010; Madadi and Abbas, 2017).

2. Materials and methods

2.1 Chemicals and other materials

Phenanthrene (^{12}C, 98%), sodium hydroxide, 2,2´-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ρ–nitrophenyl–β–D-glucopyranoside (PNG), ρ–nitrophenyl phosphate (PNP), and 3,4-dimethoxylbenzyl alcohol (veratryl alcohol) were purchased from Sigma-Aldrich, UK. [9-^{14}C] Phenanthrene (> 96%, 55.7 mCi mmol^{-1}) was acquired from American Radiolabeled Chemicals, USA. All reagents and salts for buffer solutions, phenol red, microbiological media (plate count agar and potato-dextrose agar), recipes for minimal basal salt (MBS) solution and antimicrobial agents (Amphotericin-B and Penicillin-Streptomycin-Glutamine) were obtained from Fisher Scientific, UK.

2.2 Ligninolytic fungal strains and culture conditions

Irpex lateus (CCBAS 196), *Phanerochaete chrysosporium* (CCBAS 570), *Pleurotus ostreatus* (CCBAS 473), *Trametes versicolor* (CCBAS 614), and *Bjerkandera adusta* (CCBAS 232) were obtained from the Culture Collection of Basidiomycetes (CCBAS), Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic. These strains were selected based on their abilities to delignify lignocellulosic biomass (Abdel-Hamid et al., 2013; Janusz et al., 2017). The fungal cultures were maintained on potato dextrose agar (PDA) slants (pH 5.0) at 4^0C and routinely sub-cultured every 20 days. Four actively growing mycelial plugs (0.5cm diameter) excised from one-week grown PDA agar plates were inoculated into 250 ml Erlenmeyer flasks containing 100 ml of potato dextrose broth (PDB, pH 5.0); thereafter the cultures were blended to form homogenized mycelial mats under sterile conditions for 60 seconds using a high speed Ultra-Turrax homogenizer (10,000 rpm). The blended mycelial suspensions were incubated at room temperature under a rotary shaker at 150 rpm in the dark. After 4 days of rotary incubation, 5% of mycelial pellets were further transferred to 100 ml Erlenmeyer flasks with 50 ml PDB and incubated under the same conditions as mentioned previously. Fungal pellets were harvested using a TX-40R Sorvall centrifuge (Thermo Fisher Scientific, UK) for 10 mins (3500 x *g*, 4^0C), the supernatants decarded and washed thrice with sterile distilled water (autoclaved, 121^0C). Then, the dry weights (dw) of fungal biomass were measured using the oven-dried method at 60^0C until constant weight.

2.3 Biological pre-treatment of spent brewery grains with white-rot fungi

Spent brewery grains (SBG) was used as the lignocellulosic waste material for this study. Fresh SBG was collected from Lancaster Brewery in UK, having an initial moisture content of 81% and was dried to 60% using a low-heat oven for 6 h at 60^{0}C before solid-state fermentation. Other properties of SBG are described in Table S1 (Omoni et al., 2020a). SBG (250 g ; dry matter = 40%) was transferred in sterile 1 l glass bottles and was aerated with sterile moist air then aseptically seeded with a four day old homogenised mycelial pellet (fungal strains designated, A – E) to a final dry weight of 2.5 g (0.01 g/g SBG). These flasks were incubated for 10 days (21 ± 1^{0}C) under static conditions (solid-state fermentation) for complete fungal growth, colonisation and penetration of the SBG (referred to as fungal pre-treatment).

2.4 Soil preparation, spiking and amendments with biologically pre-treated spent brewery grains

Samples of pristine agricultural soil (Dystric Cambisol) with a 2.7% organic matter was collected from a pasture field (5 – 20 cm depth) in Myerscough Agricultural College, Preston, UK. Soil microbial and physicochemical properties have been described in Couling et al., 2010 (Table S2). The soil was air-dried, thoroughly homogenised and sieved with 2mm mesh. The sieved soil was rehydrated to 60% water holding capacity (WHC) with deionized water and soil was spiked with ^{12}C-phenanthrene according to the method as previously described (Doick et al., 2003). Briefly, soil sample was spiked with ^{12}C-phenanthrene with acetone as carrier solvent to a final concentration of 100 mg/kg (dry wt), following bolus methodology and venting for 3 hr in the fume hood. Subsequently, the ^{12}C-phenanthrene spiked soil was amended with 20% of SBG. The amount of SBG added to phenanthrene spiked soil was based on our previous study (Omoni et al., 2020a). These mixtures were mixed with a stainless-steel spoon for homogeneous distribution in the soil. Soils without phenanthrene and fungal treated SBG serve as blanks. Controls included phenanthrene but lacked pre-treated SBG and abiotic controls (autoclaved-sterilized soils) to ensure that the biocatalytic activity observed in the controls was provided only by soil enzyme. The same soil moisture conditions (20%) after addition of fungal pre-treated SBG were maintained in all soil microcosms throughout the study. Soils with fungal pre-treated SBG were transferred into sterile amber bottles and incubated in the dark at 21± 1°C (n = 3) with sampling period for 1, 25, 50, 75 and 100 d soil-PAH contact time.

2.5 Influence of biological pre-treatment of spent brewery grains on the biodegradation of ^{14}C-phenanthrene in soil

Mineralisation of [9-^{14}C] phenanthrene to $^{14}CO_2$ was monitored in SBG amended soils after 1, 25, 50, 75 and 100 days soil-PAH contact time using respirometry assays, in a modified 250 ml Schott bottles (Teflon-lined screw cap) as developed and described by Reid et al. (2001) and Semple et al. (2006). The respirometry assays were prepared with soil-pre-treated SBG mixtures (10 ± 0.2 g, dw) with 30 ml of deionized water and [^{14}C] phenanthrene standard (98.2 Bq g^{-1} soil) per respirometer in 1:3 soil/water slurry (n = 3). Respirometers were shaken alongside with controls and blanks on a flat-bed orbital shaker (100 rpm) and incubated in the dark at 21 ± 1°C for 14 days with $^{14}CO_2$ traps (1 ml of 1 M NaOH). After the addition of a liquid scintillation cocktail (6 ml), ^{14}C-activity on samples were measured daily by a liquid scintillation counter (LSC) for 10 mins using standard protocols for counting and automatic quench correction (Reid et al., 2001; Macleod and Semple, 2006).

2.6. Soil physico-chemical analyses

The influence of the pre-treated SBG by the five selected basidiomycetes fungi on the carbon to nitrogen ratio (C:N) and pH level in amended soils were determined according to the method described by Wilke, 2010 and Larsson et al., 2018, respectively.

2.7. Enzyme assays

Soil enzyme activities were monitored by UV- visible spectrophotometry (Japan corporation, Japan) for ligninolytic enzymes- laccase (LAC), peroxidases: manganese peroxidase (MnP) and lignin peroxidase (LiP), as well non-ligninolytic enzymes- β-glucosidase (βGA) and acid phosphatase activities (ACP). LAC enzyme activity was assayed by 0.5 mM ABTS in 1 mM sodium acetate buffer (pH 3.0) as substrate at 420 nm (Bourbonnais and Paice, 1990 - modified). The LiP activity was determined by increasing absorbance at 310 nm, which resulted from the oxidation of 0.2mM veratryl alcohol to veratryl aldehyde in 25 mM sodium tartrate buffer, pH 3.5 (Tien and Kirk, 1988). The MnP activity in soils was monitored as previously described (Chan-Cupul et al., 2016) by the oxidation of 0.01% (wt/vol) phenol-red as substrate in sodium succinate buffer (20 mM, pH 4.5) in a reaction mixture also containing 0.1 mM $MnSO_4$, 25 mM lactate, 0.1 % (wt/vol) bovine serum albumin and 0.1 mM H_2O_2. The reaction was stopped at 30^0C by the addition of 2N NaOH and absorbance measured at 610 nm. β-glucosidase activity was measured using ρ–nitrophenyl–β–D-glucopyranoside (PNG) as substrate (Eivazi and Tabatabai, 1988). Phosphatase activity was determined using ρ–nitrophenyl phosphate (PNP) solution as substrate using the method described by (Tabatabai and Bremner, 1969). All enzyme measurements per sample were done in replicates (n = 3).

2.8. Microbial cell numbers

The indigenous microbial population (total heterotrophs and phenanthrene-degraders) was quantified through the cell numbers in fungal pre-treated SBG-amended soils at each ageing period (1, 25, 50, 75, and 100 d) using the spread plate technique as described previously (Okere et al., 2012; Omoni et al., 2020a). Microbial numbers were assessed as colony-forming units per grams soil dry weight (CFUs/g_{dw} soil, n =3).

2.9 Statistical analysis

The data were subjected to parametric paired student's t-tests and one-way ANOVA (phenanthrene spiked soil and contact time as factors) using the Statistical Package for the Social Sciences (IBM SPSS Version 25.0) followed by Tukey's post-hoc and Games-Howell tests to compare significant differences in means of samples within and across groups at 95% confidence level ($p < 0.05$). SigmaPlot 10.0 software (Systat Software Inc., USA) was used for the graphical representations of data. Pearson's correlation was used to determine and interpret the relationships between the kinetics of ^{14}C-phenanthrene mineralisation and soil biological activities in organic waste-amended soils (Omoni et al., 2020b). The Pearson's correlation coefficient (r) was ranked based on the linear association between variables on a scale that range between +1 and −1. The strength of the relationship between the two variables is either strong, weak or moderate as their absolute values approaches +1 and −1.

3. Results

3.1 Fungal pre-treated spent brewery grains on the mineralisation of ^{14}C-phenanthrene in soils

An assessment of the biological pre-treatments on the biodegradation of phenanthrene in soils was determined through biodegradation parameters such as the lag phase, fastest rate and cumulative extent of ^{14}C-phenanthrene mineralisation (Fig 1 and Table 1). In soil treatments, the results revealed significantly shorter ($p < 0.05$) lag phases for PAH amended soils. The longest and shortest lag phases were found for *T. versicolor* (3.21 ± 0.29, 1 d) and *I. lateus* (0.11 ± 0.02, 75 d), respectively in amended soils (Table 1). Compared to the non-amended soil, significantly shortened lag phases were observed in amended soils throughout the study period; in particular, contact points (1d– 50 days) showed considerably reduced lag phases ($p < 0.001$). Treatment with pre-treated SBG oz\f *P. chrysosporium* and *B. adusta* in amended soils, although not significantly different, showed also reduced lag phases when compared to the other soil conditions at 1 d incubation period. Generally, the lag phases in all of the soil conditions were statistically similar ($p > 0.05$) for most time points, but showed an average lag phase reduction of 66.8, 90.7, 90.4 and 87.8% after 25, 50, 75 and 100 d, respectively, compared to 1 d soil incubation. The reduction in the lag phase was predominant in soils amended with *T. versicolor* (96 %), closely followed by *P. ostreatus* (95.4 %) and *I. lateus* (95.4 %) after 75 d soil aging. Although the *B. adusta* and *P. chrysosporium* also showed significant reductions of 89.6 and 83.2 % in the lag phases after 50 d soil-PAH contact time, respectively. After 100 d of soil incubation, *T. versicolor* and *I. lateus*-amended soils were observed to have significantly shortened the lag phases when compared to other amended and control soils ($p < 0.05$).

The effect of the fungal pretreatment of waste materials on the fastest rates of ^{14}C-phenanthrene mineralisation (the highest % $^{14}CO_2$ per day) in amended soils was also measured at 1, 25, 50, 75 and 100 d (Fig. 1 and Table 1). At 1 d soil-PAH contact time, all soil conditions resulted in significantly faster rates ($p < 0.05$) than the control soils with exception of soil amended with *I. lateus*-pretreated SBG, which was statistically similar to the control soils (Table 1). No significant increases in the fastest rates of mineralisation were observed after 25 d between soil conditions and control ($p > 0.05$), except for soil amended with *P. chrysosporium*-pretreated SBG which showed a significant increase in the fastest rate of mineralisation ($p > 0.001$). However, after 50 d of soil incubation, there were significant increases in the rates of mineralisation ($p < 0.05$) in all amendment conditions compared to the unamended soil. Furthermore, the data showed signficant increases in fastest rates in all pre-treated SBG-amended soils after 50 d (2.83–4.52 % d^{-1}) and 75 d (2.70–3.41 % d^{-1}) with an average increase of 226 and 209%, respectively, compared to 1-d incubation. Notably, amongst the fungal pre-treated SBG investigated, *T. versicolor* showed significantly faster ($p < 0.001$) rates of 4.52, 3.41 and 2.56% d^{-1} in amended soils after 50, 75 and 100 d incubation, respectively. However, in our investigation, when compared to all fungal pre-treated SBG-amended soils, the non-amended soil showed significant increases ($p < 0.01$) in the rates of mineralisation as soil-PAH contact time increased after 75 (24.8%) and 100 d (35.8%), respectively.

The cumulative extents of ^{14}C-phenanthrene mineralisation in soils after amendments with the fungal pre-treated SBG were also assessed (Table 1 and Fig. 1). Significant extents of phenanthrene mineralisation ($p < 0.05$) were observed for all soil conditions at each contact point during the investigation (1 d–100 d). The highest extents of ^{14}C-phenanthrene mineralisation were observed at 1 d (69.7 %) soil-PAH contact time, for example, at 1 d incubation, there were higher extents of phenanthrene mineralisation (55.2 – 69.7%) in all soils' amendments compared to other contact points (25–100 d); the highest being for soil condition with *T. versicolor* (69.7 %, 1d) while the fungus *I. lateus* (29.4%, 100 d) had the least extent of phenanthrene mineralised in amended soils. Similar results were observed at longer incubation periods (25 d and 50 d) where the extents of mineralisation were also significantly higher ($p < 0.05$) in all amended conditions compared to the control soil (Table 1). Notably, at the end of the incubation period (100 d), soil amended with pre-treated SBG of *B. adusta*, followed by *T. versicolor,* showed significant increases ($p < 0.05$) in ^{14}C-phenanthrene mineralisation compared to the control soils and other soil conditions. Generally, the extent of ^{14}C-phenanthrene mineralisation for the five studied fungi in amended soils could be presented in the following order relative to control (*T. versicolor* > *B. adusta* > *P. ostreatus* > *P. chrysosporium* > *I. lateus*).

3.2 Soil physicochemical properties on ^{14}C-phenanthrene catabolism in soils

Soil pH in most of the amended conditions were not significantly different ($p > 0.05$) over time except for 25 d and 50 d, which showed significantly higher pH values ($p < 0.05$) in all amended soils investigated (Fig. S1). Higher extents of mineralisation (≥ 50%) were generally found between slightly acidic (6.3) and neutral pH (7.2). The extents of ^{14}C-phenanthrene mineralisation were positively correlated ($p < 0.05$) with soil pH in most fungal pre-treated SBG-amended soils (Fig. S5). In the case of soil C:N ratio, similar results were observed in all amended soils over a 100 d period (Fig. S2). After 1 d soil-PAH contact time, pre-treated SBG in amended soil significantly influenced the C:N ratio ($p < 0.05$) compared to control soils. However, soil pH significantly increased ($p < 0.05$) in amended soils (*T. versicolor*, *B. adusta* and *P. ostreatus*) at 1–50 d soil incubations and then significantly reduced ($p < 0.05$) in all amended conditions with increases in contact time (75–100 d). Similar increases in pH were also observed for amended soil (1 d and 50 d) with *P. chrysosporium*-pretreated SBG. Higher C:N ratios were observed for *P. chrysosporium* followed by *P. ostreatus* throughout the study period. The correlation analysis showed significantly positive correlations of pre-treated SBG of *T. versicolor*, *B. adusta* and *P. chrysosporium* with the overall extents of mineralisation in amended soils (Fig. S5).

3.3 Ligninolytic enzyme activities on ^{14}C-phenanthrene catabolism in soils

Changes in LAC activity were monitored over a 100-d soil-PAH contact time (Fig. 2 a). LAC activity levels were significantly higher ($p < 0.05$) at all amended soils in most contact points, especially at 1 d and 25 d, compared with the control (non-amended) soils. However, LAC activity were significantly reduced in all amended soil conditions ($p < 0.05$) from this contact points onwards (25–100 d). Furthermore, the LAC activity was highest (2.31 U g^{-1}, 1 d) in *T. versicolor*-amended soil. Results also showed that the *T. versicolor* soil condition displayed higher LAC activity closely followed by *P. ostreatus* and *P. chrysosporium* throughout the study period. After 100 d soil-PAH contact time, pre-treated SBG-amended soils showed no detectable LAC activity.

The LiP enzyme activity in amended soils was also assayed over time (1d–100 d) (Fig. 2 b). Significantly higher LiP activity was detected in all the amendments after 1 d incubation compared to the control soil. However, low activity levels were recorded with longer incubation period (25–50 d) and these observed reductions were significantly higher than the controls (Fig. 2, $p < 0.05$). Noticeably, there were no measurable LiP activity after 75 d and 100 d aging (Fig. 2 b The highest LiP enzyme level was observed at *P. chrysosporium* soil condition (3.50 U g^{-1}, 1 d), closely followed by *B. adusta* (2.62 U g^{-1}, 1 d) and *I. lateus* (1.42 U g^{-1}, 1 d). Overall, soils amended with both *P. chrysosporium* and *B. adusta* displayed significantly higher LiP enzyme levels in comparison with other treatment conditions, while the unamended soil showed no detectable LiP activity throughout the study period (Fig. 2 b).

MnP activity was detected for all amendment conditions in almost all time points (Fig 2 c). Compared to the other ligninolytic enzymes, MnP enzyme was best stimulated in fungal pre-treated SBG-amended soils throughout the investigation. MnP activity was also measurable in unamended soil, although these levels were significantly lower ($p < 0.05$) compared to all of the amended soils throughout the incubation period. After 1 d soil-PAH contact time, the fungal pre-treated SBG soil incubations showed significant effects on the MnP enzyme activity ($p < 0.001$). On the other hand, all amendment conditions showed significantly reduced MnP activity after 25 d soil-PAH contact time apart from *T. versicolor*-amended soil, which showed significantly higher MnP. In addition, all amendment conditions showed significantly reduced MnPs ($p < 0.05$) with extended soil-PAH contact time (50–100 d). The highest level of MnP activity was observed in soil amended with *B. adusta* (6.24 U g^{-1}) followed by *T. versicolor* (5.88 U g^{-1}) and *I. lateus* (4.26 U g^{-1}). Data revealed that soil conditions (*B. adusta* and *T. versicolor*) generally displayed higher levels of MnP in comparison to the other treatments and control throughout the study period, while *T. versicolor*-amended soil displayed the highest MnP activity (0.61 U g^{-1}) after 100 d soil-PAH contact time. Strong positive correlations ($p < 0.001$) were observed in all amended soils between the ligninolytic enzyme activities (LAC, LiP and MnP) and the lag phases as well as with the overall extents of ^{14}C-phenanthrene mineralisation, respectively; while the fastest rates of mineralisation negatively correlated with all ligninolytic enzymes studied in all of the amended soils (Table S4). Similarly, there were observed positive correlations among the three ligninolytic enzymes (LAC, LiP and MnP) in PAH contaminated soils (Table S4).

3.4. Non-ligninolytic enzyme activities on the catabolism of ^{14}C-phenanthrene in soils

Soil βGA activity were significantly increased ($p < 0.01$) by all fungal pre-treated SBG' amendments over time (1 d–100 d) compared to the control soil (Fig. 3). Furthermore, the βGA activity were increased by an average of 1.73- and 2.08-fold after 25 d and 50 d, when compared to 1 d soil-PAH contact time, respectively. The highest and lowest βGA activity levels of 12.6 U g^{-1} and 2.60 U g^{-1} in amended soils were measured for *T. versicolor* and *P. chrysosporium* after 50 d and 100 d, respectively. Generally, the βGA enzymes were stimulated in the presence of pre-treated SBG in amended soils with these orders of magnitude (*T. versicolor* > *P. chrysospsorium* > *P. ostreatus* > *B. adusta* > *I. lateus*). The βGA activity was strongly correlated with the fastest rates of mineralisation ($p < 0.001$) for most fungal pre-treated SBG amended to soil however no correlation existed with the lag phases and overall extents of ^{14}C-phenanthrene mineralisation to $^{14}CO_2$ (Table S4).

The ACP activity in amended soils were markedly influenced within and across all time points and were observed to be significantly higher than the control soils throughout the study period (Fig. 4). In comparison to all the other soil enzymes assayed, ACP were greatly stimulated by the addition of pre-treated SBG of *P. chrysosporium* showed significant ACP levels ($p < 0.05$) at 1 d soil-PAH contact time compared to other amendment conditions and control soils. After 25 d onwards, ACP activities were significantly increased in all amended soils except for *P. chrysosporium*-amended soil, which showed significantly reduced ACP activity ($p < 0.05$) with increasing soil-PAH contact time. However, after 50 d incubation, the ACP enzymes were not significantly influenced ($p > 0.05$) except for *T. versicolor*-amended soil, which exhibited a 1.25-fold increase in enzyme activity compared to 25 d incubation. Maximum ACP activity was detected for *B. adusta* (25.2 U g^{-1}, 25 d) followed by *T. versicolor* (19.2 U g^{-1}, 50 d) in amended soils, while the least ACP activity was found in amended soil with *I. lateus* (2.54 U g^{-1}, 75 d). Furthermore, ACP activities were significantly stimulated ($p < 0.05$) at the end of the incubation period (100 d) compared to 50 d and 75 d. Soil ACP activity showed positive correlations with fastest rates in *P. chrysosporium* and *P. ostreatus*-amended soils. Significant positive correlations were also found between ACP enzyme activity and lag phases ($p < 0.01$) and extents of mineralisation ($p < 0.001$), respectively, in *P. chrysosporium* only (Table S4).

3.5. Microbial quantification in fungal pre-treated SBG-amended soils

The CFUs of the microbial populations (bacteria and fungi) were significantly higher ($p < 0.01$) than the control (non-amended) soils throughout the study (1d– 100d) (Table S3). Both heterotrophic and phenanthrene-degrading microbial CFUs were significantly higher ($p < 0.05$) at all time points, while the phenanthrene-degrading bacterial numbers in amended soils increased significantly ($p < 0.05$) from 1 d to up to 50 d soil-PAH contact time. In addition, soil amended with pre-treated SBG (*T. versicolor,* 25 d, 50 d & 100 d) closely followed by *P. ostreatus* (75 d) showed higher phenanthrene-degrading bacterial CFUs as compared to other treatment conditions. In comparison to the phenanthrene-degrading fungal numbers, a significantly higher CFU was observed in *P. chrysosporium* (1 d, 75–100 d) followed by *T. versicolor* (25–50 d) as compared to other soil conditions (Table S3).

Relationships between the soil microbial numbers (bacteria and fungi) and the kinetics of ^{14}C-phenanthrene mineralisation over the 100 d period were examined (Fig. S3 and S4). Significant positive relationship ($p < 0.05$) existed between the number of phenanthrene-degrading bacteria and fastest rate of mineralisation in *T. versicolor*-amended soil only. The soil C:N ratios in amended soils with pre-treated SBG (*B. adusta*, *P. chrysosporium* and *T. versicolor*) were significantly correlated ($p < 0.05$) with phenanthrene-degrading fungal numbers. Also, we observed significant positive correlations between the phenanthrene-degrading fungal numbers with fastest rates ($p < 0.05$) and strong positive correlations with overall extents ($p < 0.05$) of ^{14}C-phenanthrene mineralisation in amended soil (*P. chrysosporium, B. adusta* and *T. versicolor*) (Fig. S3–S4).

4. Discussion

4.1 Influence of fungal pre-treated spent brewery grains on the mineralisation of ^{14}C-phenanthrene in soils

In soils, amendment with organic waste materials, in particular the lignocellulosic ones, played important roles in PAH biodegradation in contaminated soil (Novotny et al., 2000; Winquist et al., 2014; Omoni et al., 2020a). However, fungal pre-treatment of lignocellulosic material, such as SBG, increased the accessibility of available sugar monomers and other nutrients for microbial growth and activity. In this study, we found that all fungal pre-treated SBG resulted in shorter lag phases with increases in the rates and extents of mineralisation of ^{14}C-phenanthrene in amended soils; however, the fungal strains showed different PAH biodegradation kinetics within and at each of the sampling time points.

This study showed that the addition of pre-treated SBG of *T. versicolor* to soil greatly reduced the lag phase of ^{14}C-phenanthrene mineralisation, while soil amended with the fungus *I. lateus* was the least influenced. Although there were variations in the lag phase in amended soils, greatest reductions were observed after 75 d and 100 d soil incubations, indicating that increases in soil-PAH contact time led to increase microbial adaptation and activities which might be due to increase in available microbial nutrients and higher released of sugars (glucose) in pre-treated SBG-amended soils. Also, this reduced lag phases may be attributed to the increased survival rates of the fungal mycelia in pre-treated SBG (Czaplicki et al., 2018) and the extent of PAHs-interaction with soil matrices (Wang et al., 2012; Oyelami et al., 2015). In particular, the significantly shorter lag phases displayed by *T. versicolor* compared to other amendment conditions suggests a higher metabolism of SBG as carbon and energy source for PAH dissipation (Han et al., 2017); faster adaptation to phenanthrene and possible synergistic interactions with soil indigenous microorganisms (Kästner and Miltner, 2016; Omoni et al., 2020a). However, the overall findings suggest that all fungal pre-treated SBG led to increased microbial adaptation to the target contaminant and consequently the reduction in the lag phases in amended soils. Omoni et al. (2020b) observed that the lag phases were longer in soil amended with SBG without pre-treatment at same phenanthrene concentration when compared to the present work. The authors also reported the shortest and longest lag phases for 20% SBG were 0.21 d and 3.50 d as compared to our study (0.11 d and 3.21 d) in amended soils after SBG pre-treatment, respectively.

The fastest rates of ^{14}C-phenanthrene mineralised to $^{14}CO_2$ depended on the fungal strain employed in the pre-treatment of SBG amended to soil in the present study. This may be associated with the initial preferential attack by fungi for lignin to enriched cellulose, fungal interaction with soil matrix and other soil microbes, and mycelial tolerance to PAH toxicity (Ghosal et al., 2016; Akhtar and Mannan, 2020). The data showed that all amended soils had significantly faster rates of mineralisation at both 1 and 50 d incubations, indicating the potential of the fungal inoculants for induction of PAH catabolism (Andriani and Tachibana, 2016). However, the rates of mineralisation were negatively affected with extended soil-PAH interaction (75 d and 100 d). This may have resulted from increased sorption of PAH into soil matrices and possibly less PAH partitioning, and decrease in the bioavailability of rapidly desorbable PAH fraction to microbial cells in amended soils (Semple et al., 2003; Cui et al., 2013). Consequently, this may hinder the migration capacity of enzyme to reach contaminant sorption site within soil pore for PAH biodegradation. In addition, contaminant concentration and properties, soil properties and contact time, and microbial dynamics may also be attributed (Omoni, et al., 2020b). It was also observed that SBG-associated *T. versicolor* had significantly faster rates of mineralisation in amended soils compared to the other amendment conditions after 50 d and 75 d of soil incubations, which is evident in the strong positive correlations that existed between phenanthrene-degraders (bacteria and fungi) and rates of mineralisation in *T. versicolor*-amended soil. This clearly indicated that this fungus (*T. versicolor*) has shown the greatest ability to improve the rate of PAH mineralisation in amended soil.

Many white-rot fungi are mycoremediators for PAH metabolism in impacted PAH contaminated soils and have the catabolic function to transform toxic organic chemicals to less toxic compounds or CO_2 (Field et al., 1992; Novotný et al., 1999). In this study, we observed significantly higher extents of mineralisation for all fungal pre-treated SBG amended soils in most contact points. Soils amended with pre-treated SGB facilitated the amounts of ^{14}C-phenanthrene mineralised in the present study. Here, it can be hypothesised that there was a (i) higher transport of phenanthrene contaminant by cytoplasmic streaming to the mycelial network (fungal pipelines), and (ii) higher diffusion of phenanthrene to cells in amended soils (Furuno et al., 2012). It should be noted that greater extents of mineralisation were measured at most time points but in all cases the results depend on the fungal strain used in the pre-treatment of SBG in amended soil. In particular, the pre-treated SBG of *T. versicolor* and *B. adusta* were the most efficient fungal strains with higher extents of mineralisation in amended soils compared to the other amendment conditions in almost all time points. The higher levels of mineralisation of ^{14}C-phenenthrene by both fungi (*B. adusta* and *T. versicolor*) can also be attributed to the high secretion of ligninolytic enzymes in amended soils, especially the MnPs (Lladó et al., 2013; Andriani and Tachibana, 2016). Particularly, previous studies have revealed that *T. versicolor* has the capacity to synthesize extracellular LiP, MnP and laccase (Schlosser et al., 1997; Hossain and Anantharaman, 2006), which also can degrade PAHs (Bamforth and Singleton, 2005). Consequently, it is suggested that these two fungal strains in amended soils possess higher capacity for PAH biodegradation (Peng et al., 2008). Furthermore, the pre-treatment step with fungal strains improved (by 8–21%) the efficiency of ^{14}C-phenanthrene mineralisation in amended soils when compared to our previous study without fungal pre-treatment (Omoni et al., 2021b). Thus, it may also be hypothesised that all fungal strains used in the pre-treatment of SBG had positive influence on the microbial activities (bacteria and fungi) in amended soils. This is further confirmed by the positive correlations observed in most amended soils between the phenanthrene-degraders (especially fungi) and mineralisation (rates and extents).

4.2 The influence of ligninolytic enzymes on ^{14}C-phenanthrene catabolism in soils

Previous studies have showed that the degradation of PAHs in contaminated soils by white-rot fungi depends on ligninolytic enzymes secretion in soil (Pozdnyakova, 2012; Kadri et al., 2017). Soil amended with fungal pre-treated SBG increased all ligninolytic enzyme activities in this study. However, the fungal strains behaved differently in the secretion of ligninolytic enzymes in amended soils, indicating differences in their enzyme capacity, release and complexity in soil. Fungal pre-treated SBG of *T. versicolor* followed by *P. chrysosporium* in amended soils showed higher levels of LAC than the other amended conditions, indicating the potential of these fungi as proficient LAC inducers in contaminated soil. Similar soil-based studies have reported increases in LAC activity of *T. versicolor* (Lang et al., 1998; Novotný et al., 1999). However, few studies have reported very low levels of LAC activity with *P. chrysosporium* in soil spiked with organic chemicals (Fragoeiro and Magan, 2008; Yu et al., 2011). This work clearly shows that the LAC production by *P. chrysosporium* can be induced in soil amended with fungal pre-treated SBG. On the other hand, the results showed that LiP activities were greatly influenced by *P. chrysosporium* followed by *B. adusta* compared to other fungal strains in amended soils. It has been shown that these two white-rot fungi, particularly *P. chrysosporium,* are key producers of LiPs in the biodegradation of PAHs in lignocellulose-amended soils (Andriani and Tachibana, 2016; Kadri et al., 2017), thereby indicating that the two fungal strains are more efficient LiP producers in PAH contaminated soil in the present study. MnP was the most secreted in amended soils in the present study. This observation of higher levels of MnP enzymes is consistent with the findings of Novotný et al. (2004) and Pozdnyakova (2012), who reported significant role of MnPs in the degradation of recalcitrant compounds in soil by similar fungal strains in our study. Although, other fungal enzymes, including cytochrome P450 monooxygenase are also involved in PAH degradation (Durairaj et al., 2016). In our experiments the high levels of MnPs in amended soils, with both *B. adusta* and *T. versicolor* pre-treated SBG, indicated the higher potential of these fungi for the stimulation of MnPs (Lladó et al., 2013; Andriani and Tachibana, 2016).

4.3. The influence of non-ligninolytic enzymes on the catabolism of ^{14}C-phenanthrene in soils

Both βGA and ACP activities significantly improved in all PAH-spiked soils following fungal pre-treated SBG amendments. Previous research has also demonstrated increases in these hydrolytic enzymes βGA and ACP) resulting from the degradation of PAH in contaminated soil (Adetunji et al., 2017; Košnář et al., 2019; Lipińska et al., 2019). In addition, the high production of βGA and ACP observed in the amended soils, which are potential bio-indicator systems of soil quality and health of a degraded and contaminated soils (Dindar et al., 2015; Chang et al., 2017), suggested increased carbon and energy (organic matter and nutrients), and a subsequent increased in microbial growth and activities in all amended soils (especially *T. versicolor* and *B. adusta*). We also found that both βGA and

ACP were detected in amended soils as the overall extents of ^{14}C-phenanthrene mineralised decreased over time. As a consequence, these enzymes were not affected by either PAH toxicity or increases in soil-PAH contact time. Although the potential roles of both enzymes (βGA and ACP) in PAH degradation is still unknown; their presence in soil after an organic amendment is very helpful not only for soil remediation but also in soil biology.

5. Conclusions

This investigation demonstrated that the biological pre-treatment of lignocellulosic waste material such as the spent brewery grains, has the potential to be used as a cost-effective and sustainable remedial method for enhancing the biodegradation of organic contaminants in soil. Assessment of the soil conditions showed that the fungal pre-treated SBG influenced the mineralisation of ^{14}C-phenanthrene, stimulate the enzyme activities, and microbial population in all amended soils; however, these primarily depended on the fungal strain used for the pre-treatment of SBG before soil amendment. At most of the sampling time points, reductions in lag phases, faster rates and greater extents of ^{14}C-phenanthrene mineralisation were found in all fungal pre-treated SBG-amended soils (especially *T. versicolor* and *B. adusta*). This study showed that *T. versicolor* and *B. adusta* are more efficient degraders of the PAH in the soil. However, in most cases *T. versicolor* followed by *B. adusta* and *P. chrysosporium* displayed higher levels of both soil enzyme activities investigated (ligninolytic and non-ligninolytic). Overall, the ligninolytic enzymes generally decreased, while non-ligninolytic enzymes increased as the extent of mineralisation diminished in all amended soils over time. The study demonstrated that fungal pre-treatment of organic waste materials provide a promising approach for *in situ* and enhanced bioremediations of organic contaminants in soil.

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References

Abdel-Hamid, A.M., Solbiati, J.O., Cann, I.K.O., 2013. Insights into lignin degradation and its potential industrial applications. Adv. Appl. Microbiol. 82, 1–28.

Akhtar, N., Mannan, M.A., 2020. Mycoremediation: Expunging environmental pollutants. Biotechnol. Rep. 26, e00452.

Adetunji, A.T., Lewu, F.B., Mulidzi, R., Ncube, B., 2017. The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators : a review. J. Plant Sci. Plant Nutr. 17, 794–807.

Al-Hawash, A.B., Dragh, M.A., Li, S., Alhujaily, A., Abbood, H.A., Zhang, X., Ma, F., 2018. Principles of microbial degradation of petroleum hydrocarbons in the environment. Egypt. J. Aquat. Res. 44, 71–76.

Alvim, G.M., Pontes, P.P., 2018. Aeration and sawdust application effects as structural material in the bioremediation of clayey acid soils contaminated with diesel oil. Int. Soil Water Conserv. Res. 6, 253–260.

Andriani, A., Tachibana, S., 2016. Lignocellulosic materials as solid support agents for *Bjerkandera adusta* SM46 to enhance polycyclic aromatic hydrocarbon degradation on sea sand and sea water media. Biocatal. Agric. Biotechnol. 8, 310–320.

Anza, M., Salazar, O., Epelde, L., Becerril, J.M., Alkorta, I., Garbisu, C., 2019. Remediation of organically contaminated soil through the combination of assisted phytoremediation and bioaugmentation. Appl. Sci. 9, 4757.

Baldrian, P., 2006. Fungal laccases-occurrence and properties. FEMS Microbiol. Rev. 30, 215–242.

Bamforth, S.M., Singleton, I., 2005. Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. J. Chem. Technol. Biotechnol. Int. Res. Process Environ. Clean Technol. 80, 723–736.

Baruah, J., Nath, B.K., Sharma, R., Kumar, S., Deka, R.C., Baruah, D.C., Kalita, E., 2018. Recent trends in the pretreatment of lignocellulosic biomass for value-added products. Front. Energy Res. 6, 141.

Bourbonnais, R., Paice, M.G., 1990. Oxidation An expanded of non-phenolic substrates role for lactase in lignin biodegradation. FEBS Lett. 267, 99–102.

Brändli, R.C., Bucheli, T.D., Kupper, T., Furrer, R., Stadelmann, F.X., Tarradellas, J., 2005. Persistent Organic Pollutants in Source-Separated Compost and Its Feedstock Materials-A Review of Field Studies. J. Environ. Qual. 34, 735–760.

Breedveld, G.D., Sparrevik, M., 2000. Nutrient-limited biodegradation of PAH in various soil strata at a creosote contaminated site. Biodegradation 11, 391–399.

Chae, Y., Cui, R., Kim, S.W., An, G., Jeong, S-W., An, Y-J., 2017. Exoenzyme activity in contaminated soils before and after soil washing: ß- glucosidase activity as a biological indicator of soil health. Ecotoxicol. Environ. Saf. 135, 368–374.

Chan-Cupul, W., Heredia-Abarca, G., Rodríguez-Vázquez, R., 2016. Atrazine degradation by fungal co-culture enzyme extracts under different soil conditions. J. Environ. Sci. Health Pestic. Food Contam. Agri. Wastes 51, 298–308.

Chang, E-H., Chung, R-S., Tsai, Y-H., 2017. Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. Soil Sci. Plant Nutr. 53, 132–140.

Chojnacka, K., Moustakas, K., Witek-Krowiak, A., 2020. Bio-based fertilizers: A practical approach towards circular economy. Bioresour. Technol. 295, 122223.

Couling, N.R., Towell, M.G., Semple, K., 2010. Biodegradation of PAHs in soil: Influence of chemical structure, concentration and multiple amendment. Environ. Pollut. 158, 3411–3420.

Cui, X., Mayer, P., Gan, J., 2013. Methods to assess bioavailability of hydrophobic organic contaminants : Principles , operations, and limitations. Environ. Pollut. 172, 223–234.

Czaplicki, L.M., Dharia, M., Cooper, E.M., Ferguson, P.L., Gunsch, C.K., 2018. Evaluating the mycostimulation potential of select carbon amendments for the degradation of a model PAH by an ascomycete strain enriched from a superfund site. Biodegradation 29, 463–471.

Dindar, E., Topaç Şağban, F.O., Başkaya, H.S., 2015. Variations of soil enzyme activities in petroleum-hydrocarbon contaminated soil. Int. Biodeter. Biodegrad. 105, 268–275.

Doick, K.J., Lee, P.H., Semple, K.T., 2003. Assessment of spiking procedures for the introduction of a phenanthrene-LNAPL mixture into field-wet soil. Environ. Pollut. 126, 399–406.

Dotaniya, M.L., Aparna, K., Dotaniya, C.K., Singh, M., Regar, K.L., 2019. Role of soil enzymes in sustainable crop production. Enzyme Food Biotechnol. 569–589.

Durairaj, P., Hur, J.S., Yun, H., 2016. Versatile biocatalysis of fungal cytochrome P450 monooxygenases. Microb. Cell Fact. 15, 125.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606.

Field, J.A., De Jong, E., Costa, G.F., De Bont, J.A.M., 1992. Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white rot fungi. Appl. Environ. Microbiol. 58, 2219–2226.

Fragoeiro, S., Magan, N., 2008. Impact of Trametes versicolor and Phanerochaete chrysosporium on differential breakdown of pesticide mixtures in soil microcosms at two water potentials and associated respiration and enzyme activity. Int. Biodet. Biodegrad. 62, 376–383.

Futa, B., Bielińska, E.J, Ligęza, S., Chmielewski, S., Wesołowska, S., Patkowski, K., Mocek-Płóciniak, A., 2017. Enzymatic activity and content of polycyclic aromatic hydrocarbons (PAHs) in soils under low-stack emission in Lublin. Pol. J. of Soil Sci. 50, 63–74.

Furuno, S., Foss, S., Wild, E., Jones, K.C., Semple, K.T., Harms, H., Wick, L.Y., 2012. Mycelia promote active transport and spatial dispersion of polycyclic aromatic hydrocarbons. Environ. Sci. Technol. 46, 5463−5470.

Gao, D., Du, L., Yang, J., Wu, W., Liang, H., 2010. A critical review of the application of white rot fungus to environmental pollution control. Crit. Rev. Biotechnol. 30, 70–77.

Ghosal, D., Ghosh, S., Dutta, T. K., Ahn, Y., 2016. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. Front. Microbiol. 7, 1369.

Han, X., Hu, H., Shi, X., Zhang, L., He, J., 2017. Effects of different agricultural wastes on the dissipation of PAHs and the PAH-degrading genes in a PAH-contaminated soil. Chemosphere 172, 286–293.

Hatakka, A., Hammel, K.E., 2010. Fungal biodegradation of lignocelluloses. Hofrichter, M. (Ed.), The Mycota X Industrial Applications (2nd edn), Springer-Verlag Berlin, Heidelberg, pp. 319–340.

Hossain, S.M., Anantharaman, N., 2006. Activity enhancement of ligninolytic enzymes of Trametes versicolor with bagasse powder. Afr. J. Biotechnol. 5, 189–194.

Ibeto, C., Omoni, V., Fagbohungbe, M., Semple, K. 2020. Impact of digestate and its fractions on mineralization of 14C-phenanthrene in aged soil. Ecotoxicol. Environ. Saf. 195, 110482.

Idowu, O., Semple, K.T., Ramadass, K., O'Connor, W., Hansbro, P., Thavamani, P., 2019. Beyond the obvious: Environmental health implications of polar polycyclic aromatic hydrocarbons. Environ. Int. 123, 543–557.

Isroi, I., Ria, M., Syamsiah, S., Niklasson, C., Cahyanto, C., Lundquist, K., Taherzadeh, M.J., 2011. Biological treatment of lignocelluloses with white-rot fungi and its applications: review. Bioresources 6, 5224–5259.

Janusz, G., Pawlik, A., Sulej, J., Świderska-Burek, U., Jarosz-Wilkolazka, A., Paszczyński, A., 2017. Lignin degradation: Microorganisms, enzymes involved, genomes analysis and evolution. FEMS Microbiol. Rev. 41, 941–962.

Kadri, T., Rouissi, T., Brar, S.K., Cledon, M., Sarma, S., Verma, M., 2017. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review. J. Environ. Sci. 51, 52–74.

Kästner, M., Miltner, A., 2016. Application of compost for effective bioremediation of organic contaminants and pollutants in soil. Appl. Microbiol. Biotechnol. 100, 3433–3449.

Košnář, Z., Částková, T., Wiesnerová, L., Praus, L., Jablonský, L., Koudela, M., Tlustoš, P., 2019. Comparing the removal of polycyclic aromatic hydrocarbons in soil after different bioremediation approaches in relation to the extracellular enzyme activities. J. Environ. Sci. 76, 249-258.

Lang, E., Nerud, F., Zadrazil, F., 1998. Production of ligninolytic enzymes by *Pleurotus* sp. and *Dichomitus squalens* in soil and lignocellulose substrate as influenced by soil microorganisms. FEMS Microbiol. Lett. 167, 239–244.

Larney, F.J., Angers, D.A., 2012. The role of organic amendments in soil reclamation: A review. Can. J. Soil Sci. 92, 19–38.

Leonowicz, A., Matuszewska, Luterek, J., Ziegenhagen, D., Wojtas´-Wasilewska, M., Cho, N-S., Hofrichter, M., Rogalski, J., 1999. Biodegradation of lignin by white rot fungi. Fungal Genet. Biol. 27(2–3), 175–185.Lladó, S., Gràcia, E., Solanas, A M., Viñas, M., 2013. Fungal and bacterial microbial community assessment during bioremediation assays in an aged creosote-polluted soil. Soil Biol. Biochem. 67, 114–123.

Lladó, S., Solanas, A.M., de Lapuente, J., Borràs, M., Viñas, M., 2012. A diversified approach to evaluate biostimulation and bioaugmentation strategies for heavy-oil-contaminated soil. Sci. Total Environ. 435–436, 262–269.

Lipińska, A., Kucharski, J., Wyszkowska, J., 2019. Activity of phosphatases in soil contaminated with PAHs. Water, Air, Soil Pollut. 230, 298. https://doi.org/10.1007/s11270-019-4344-1.

Lukić, B., Panico, A., Huguenot, D., Fabbricino, M., van Hullebusch, E.D., Esposito, G., 2016. Evaluation of PAH removal efficiency in an artificial soil amended with different types of organic wastes. EuroMediterr. J. Environ. Integr. 1, 5.

Macleod, C.J.A., Semple, K.T., 2006. The influence of single and multiple applications of pyrene on the evolution of pyrene catabolism in soil. Environ. Pollut.139, 455–460.

Madadi, M., Abbas, A., 2017. Lignin degradation by fungal pretreatment: a review. J. Plant Pathol. Microbiol. 8, 398.

Mohammadi, A., Nasernejad, B., 2009. Enzymatic degradation of anthracene by the white rot fungus *Phanerochaete chrysosporium* immobilized on sugarcane bagasse. J. Hazard. Mater. 161, 534–537.

Novotný, Č., Erbanová, P., Šašek, V., Kubátová, A., Cajthaml, T., Lang, E., Krahl, J., Zadražil, F., 1999. Extracellular oxidative enzyme production and PAH removal in soil by exploratory mycelium of white rot fungi. Biodegrad. 10, 159–168.

Novotný, Č., Erbanová, P., Cajthaml, T., Rothschild, N., Dosoretz, C., Šašek, V., 2000. *Irpex lacteus*, a white rot fungus applicable to water and soil bioremediation. Appl. Microbiol. Biotechnol. 54, 850–853.

Novotný, Č., Svobodová, K., Erbanová, P., Cajthaml, T., Kasinath, A., Lang, E., Šašek, V., 2004. Ligninolytic fungi in bioremediation: Extracellular enzyme production and degradation rate. Soil Biol. Biochem. 36, 1545–1551.

Okere, U.V, Cabrerizo, A., Dachs, J., Jones, K.C., Semple, K.T., 2012. Biodegradation of phenanthrene by indigenous microorganisms in soils from Livingstone Island, Antarctica. FEMS Microbiol. Lett. 329, 66–77.

Omoni, V.T., Baidoo, P.K., Fagbohungbe, M.O., Semple, K.T., 2020a. The impact of enhanced and non-enhanced biochars on the catabolism of ^{14}C-phenanthrene in soil. Environ. Technol. Innov. 20, 101146.

Omoni, V.T., Lag-Brotons, A.J., Semple, K.T., 2020b. Impact of organic amendments on the development of ^{14}C-phenanthrene catabolism in soil. Int. Biodeter. Biodegrad. 151, 104991.

Oyelami, A.O., Ogbonnaya, U., Muotoh, C., Semple, K.T., 2015. Impact of activated carbon on the catabolism of 14C-phenanthrene in soil. Environ. Sci. Process. Impacts, 17, 1173–1181.

Peng, R.H., Xiong, A.S., Xue, Y., Fu, X.Y., Gao, F., Zhao, W., Tian, Y.S., Yao, Q.H., 2008. Microbial biodegradation of polyaromatic hydrocarbons. FEMS Microbiol. Rev. 32, 927–955.

Pozdnyakova, N.N., 2012. Involvement of the ligninolytic system of white-rot and litter-decomposing fungi in the degradation of polycyclic aromatic hydrocarbons. Biotechnol. Res. Int. 20, Article e243217.

Ramarajan, R., Manohar, C.S., 2017. Biological pretreatment and bioconversion of agricultural wastes, using ligninolytic and cellulolytic fungal consortia. Bioremediat. J. 21, 89–99.

Reid, B.J., Macleod, C.J.A., Lee, P.H., Morriss, A.W.J., Stokes, J.D., Semple, K.T., 2001. C-respirometric method for assessing microbial catabolic potential and contaminant bioavailability. 196, 141–146. FEMS Microbiol. Lett. 196, 141–146.

Ren, X., Zeng, G., Tang, L., Wang, J., Wan, J., Liu, Y., Yu, J., Yi, H., Ye, S., Deng, R., 2018. Sorption, transport and biodegradation – An insight into bioavailability of persistent organic pollutants in soil. Sci. Total Environ. 610–611, 1154–1163.

Riffaldi, R., Levi-Minzi, R., Cardelli, R., Palumbo, S., Saviozzi, A., 2006. Soil biological activities in monitoring the bioremediation of diesel oil-contaminated soil. Water, Air, and Soil Pollut. 170, 3–15.

Ros, M., Rodríguez, I., García, C., Hernández, T., 2010. Microbial communities involved in the bioremediation of an aged recalcitrant hydrocarbon polluted soil by using organic amendments. Bioresour. Technol. 101, 6916–6923.

Rouches, E., Herpoël-Gimbert, I., Steyer, J.P., Carrere, H., 2016. Improvement of anaerobic degradation by white-rot fungi pretreatment of lignocellulosic biomass: A review. Renew. Sustain. Energy Rev. 59, 179–198.

Sari, A., Kristiani, A., Tachibana, S., Sudiyani, Y., Abimanyu, H., 2014. Mechanisms and optimization of oil palm empty fruit bunch as a pre-grown source for white-rot fungus to degrade DDT. J. Environ. Chem. Eng. 2, 1410–1415.

Saritha, M., Anju, M.S., Lata, A., 2012. Biological pretreatment of lignocellulosic substrates for enhanced delignification and enzymatic digestibility. Indian J. Microbiol. 52, 122–130.

Schlosser, D., Grey, R., Fritsche, W., 1997. Patterns of ligninolytic enzymes in Trametes versicolor. Distribution of extra-and intracellular enzyme activities during cultivation on glucose, wheat straw and beech wood. Appl. Microbiol. Biotechnol. 47, 412–418.

Semple, K.T., Dew, N.M., Doick, K.J., Rhodes, A.H., 2006. Can microbial mineralization be used to estimate microbial availability of organic contaminants in soil? Environ. Pollut. 140, 164–172.

Semple, K.T., Doick, K.J., Wick, L.Y., Harms, H., 2007. Microbial interactions with organic contaminants in soil: Definitions, processes and measurement. Environ. Pollut. 150, 166–176.

Semple, K.T., Morriss, A.W.J., Paton, G.I., 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. Eur. J. Soil Sci. 54, 809–818.

Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307.

Tejada, M., Gonzalez, J.L., Hernandez, M.T., Garcia, C., 2008. Application of different organic amendments in a gasoline contaminated soil: Effect on soil microbial properties. Bioresour. Technol. 99, 2872–2880.

Tien, M., Kirk, T.K., 1988. Lignin peroxidase of *Phanerochaete chrysosporium*. Methods Enzymol. 161, 238–249.

Wagner, A.O., Janetschek, J., Illmer, P., 2018. Using Digestate Compost as a Substrate for Anaerobic Digestion. Chem. Eng. Technol. 41, 747–754.

Wali, A., Gupta, M., Gupta, S., Sharma, V., Salgotra, R.K., Sharma, M., 2020. Lignin degradation and nutrient cycling by white rot fungi under the influence of pesticides. 3 Biotech. 10, 1–7.

Wan, C., Li, Y., 2010. Microbial pretreatment of corn stover with Ceriporiopsis subvermispora for enzymatic hydrolysis and ethanol production. Bioresour. Technol. 101, 6398–6403.

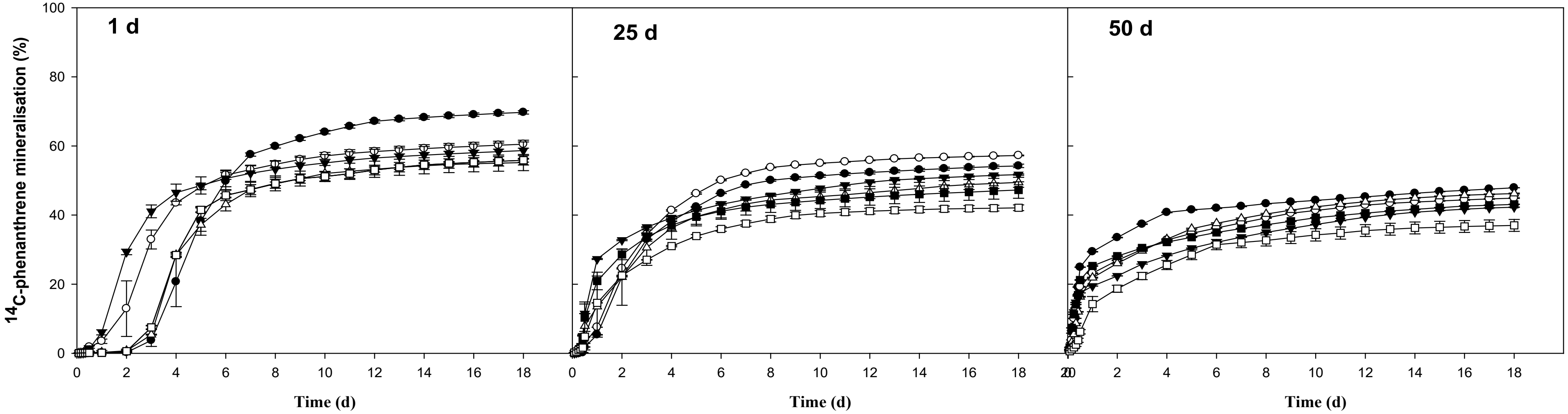
Wang, Z., Chen, S., Xu, Y., Tang, J., 2012. Aging effects on sorption-desorption behaviors of PAHs in different natural organic matters. J. Colloid Interface Sci. 382, 117–122.

Wilson, S.C., Jones, K.C., 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environ. Pollut. 81, 229–249.

Winquist, E., Björklöf, K., Schultz, E., Räsänen, M., Salonen, K., Anasonye, F., Cajthaml, T., Steffen, K.T., Jørgensen, K.S., Tuomela, M., 2014. Bioremediation of PAH-contaminated soil with fungi - From laboratory to field scale. Int. Biodeter. Biodegrad. 86, 238–247.

Yu, Z., Zeng, G.M., Chen, Y.N., Zhang, J.C., Yu, Y., Li, H., Liu, Z-F., Tang, L., 2011. Effects of inoculation with Phanerochaete chrysosporium on remediation of pentachlorophenol-contaminated soil waste by composting. Process Biochem. 46, 1285–1291.

Zhang, X.-X., Cheng, S.-P., Zhu, C.-J., Sun, S.-L., 2006. Microbial PAH-degradation in soil: degradation pathways and contributing factors. Pedosphere 16, 555–556.


1 d
25 d
50 d
^{14}C-phenanthrene mineralisation (%)
Time (d)

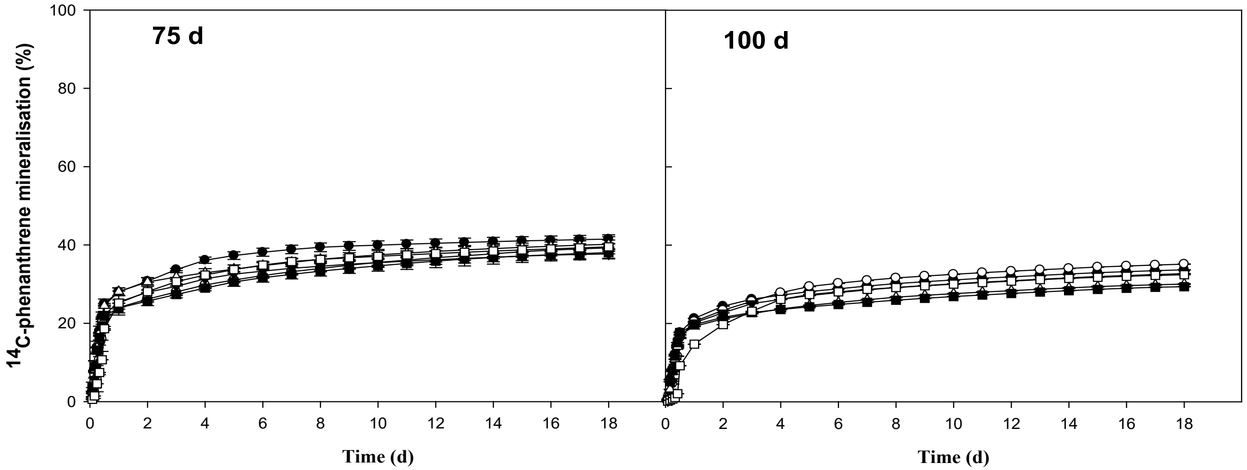

75 d
100 d
^{14}C-phenanthrene mineralisation (%)
Time (d)

Fig. 1. Development of ^{14}C-phenanthrene catabolism in soils amended with fungal pre-treated spent brewery grains after 1, 25, 50, 75, and 100d soil-phenanthrene contact time. Fungal pre-treatment: (●) *T. versicolor*, (○) *B. adusta*, (▼) *P. chrysosporium*, (△) *P. ostreatus*, (■) *I. lateus,* and control (unamended)(□). Values are mean ± SE (n = 3).

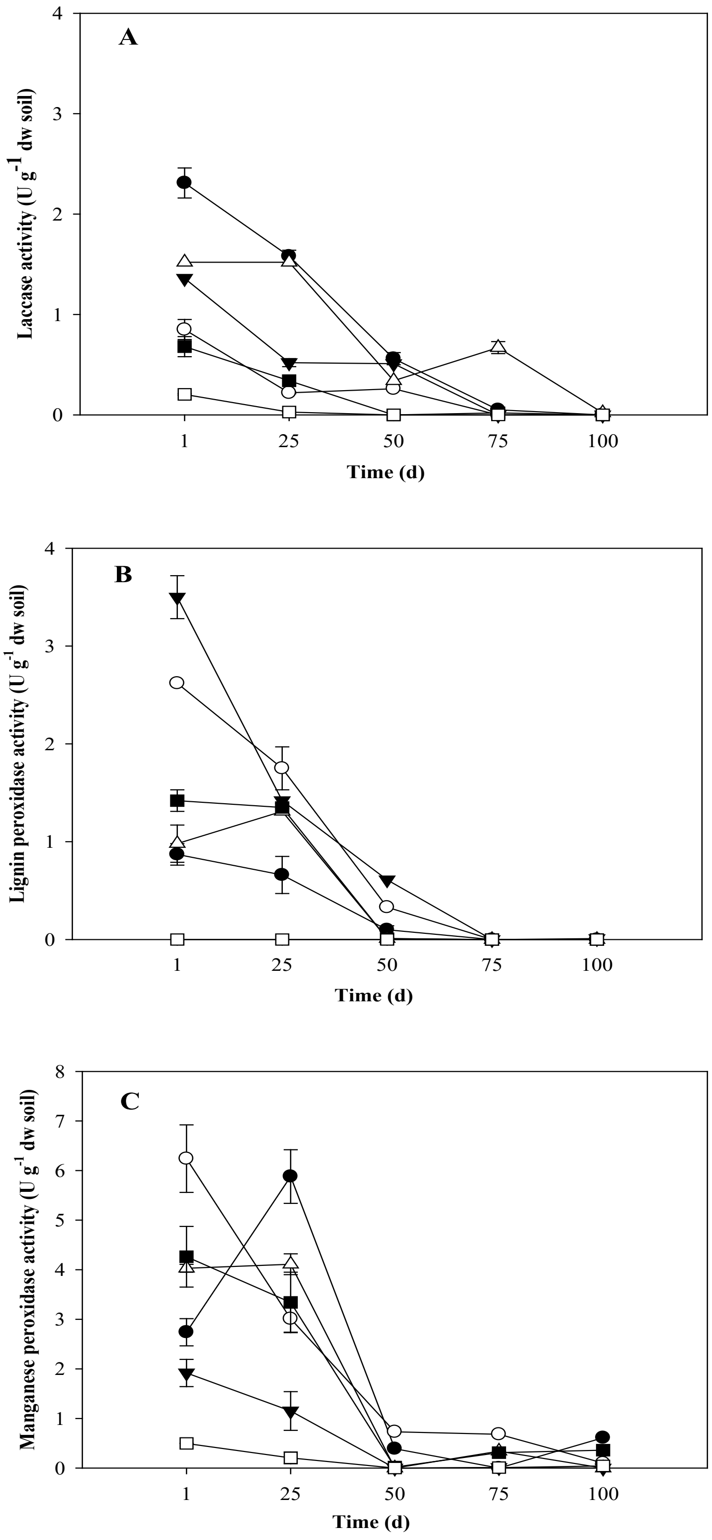


Fig. 2. Ligninolytic enzyme activities in soils amended with fungal pre-treated spent brewery grains after 1, 25, 50, 75, and 100d soil-phenanthrene contact time. **A** = Laccase (LAC) activity in amended soils; **B** = Lignin peroxidase activity (LiP) in amended soils; **C** =

Manganese peroxidase (MnP) activity in amended soils. Fungal pre-treatment: (●) *T. versicolor*, (○) *B. adusta*, (▼) *P. chrysosporium*, (△) *P. ostreatus*, (■) *I. lateus,* and control (unamended)(□). Values are mean ± SE (n = 3).

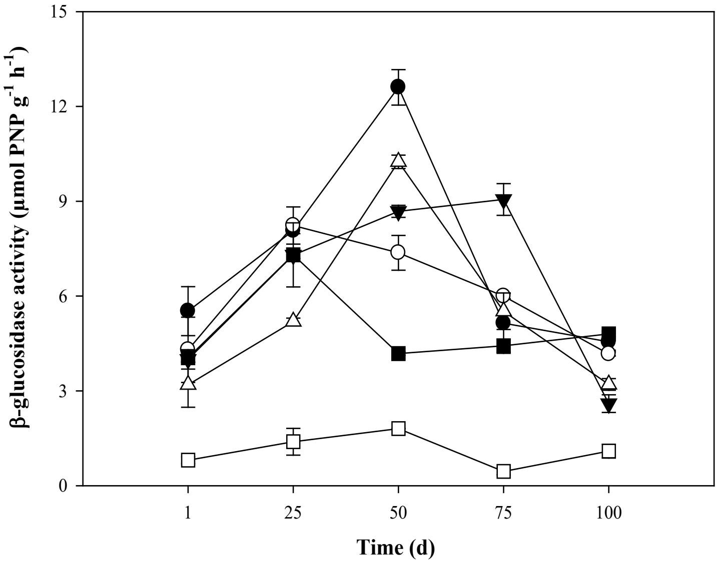


Fig. 3. Level of β-glucosidase (μmol PNG g^{-1} h^{-1}) activity in amended soils with fungal pre-treated spent brewery grains after 1, 25, 50, 75, and 100d soil-phenanthrene contact time. Fungal pre-treatment: (●) *T. versicolor*, (○) *B. adusta*, (▼) *P. chrysosporium*, (△) *P. ostreatus*, (■) *I. lateus,* and control (unamended)(□). Values are mean ± SE (n = 3).

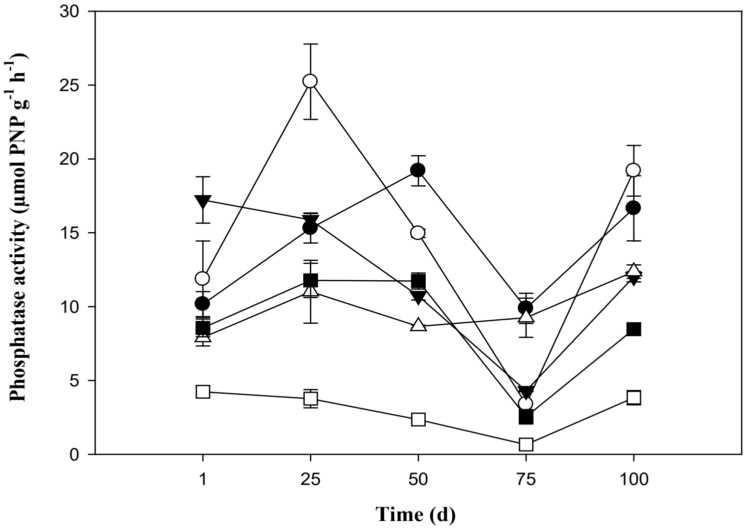


Fig. 4. Level of phosphatase (µmol PNP g^{-1} h^{-1}) activity in amended soils with fungal pre-treated spent brewery grains after 1, 25, 50, 75, and 100d soil-phenanthrene contact time. Fungal pre-treatment: (●) *T. versicolor*, (○) *B. adusta*, (▼) *P. chrysosporium*, (△) *P. ostreatus*, (■) *I. lateus,* and control (unamended)(□). Values are mean ± SE (n = 3)

Table 1. Kinetics of ^{14}C-phenanthrene in soil amended with fungal pre-treated spent brewery grains of five genera of ligninolytic fungi after 14 days respirometric assay. Values are mean ± standard error (n = 3).

Contact time (d)	Ligninolytic fungi treatment	Lag phase (d)	Fastest rate ($\%^{14}CO_2\ d^{-1}$)	Cumulative Extent (%)	Increase in extent relative to control (%)
1	*T. versicolor*	3.21 ± 0.29	0.95 ± 0.03	69.73 ± 0.49	42.3
	B. adusta	1.73 ± 0.32	1.04 ± 0.03	60.51 ± 1.16	33.5
	P. chrysosporium	0.89 ± 0.08	0.97 ± 0.01	58.66 ± 2.49	31.4
	P. ostreatus	2.92 ± 0.03	0.96 ± 0.03	55.20 ± 2.34	27.1
	I. lateus	2.65 ± 0.04	0.89 ± 0.03	55.85 ± 1.36	27.9
	Control	6.12 ± 0.39	0.79 ± 0.01	40.27 ± 0.75	0.00
25	*T. versicolor*	0.95 ± 0.02	0.83 ± 0.00	54.24 ± 0.48	22.4
	B. adusta	0.73 ± 0.01	0.73 ± 0.02	57.26 ± 0.10	26.5
	P. chrysosporium	0.42 ± 0.02	3.24 ± 0.00	51.73 ± 0.20	18.6
	P. ostreatus	0.89 ± 0.34	2.74 ± 2.09	49.47 ± 1.45	14.9
	I. lateus	0.44 ± 0.03	3.19 ± 1.39	47.25 ± 2.38	10.9
	Control	2.56 ± 0.34	0.70 ± 0.01	42.10 ± 0.61	0.00
50	*T. versicolor*	0.21 ± 0.02	4.52 ± 0.03	47.90 ± 0.01	22.8
	B. adusta	0.18 ± 0.00	3.11 ± 0.04	44.90 ± 0.10	17.6
	P. chrysosporium	0.15 ± 0.00	3.04 ± 0.01	42.26 ± 0.05	12.5
	P. ostreatus	0.23 ± 0.01	2.17 ± 0.03	46.22 ± 0.13	20.0
	I. lateus	0.13 ± 0.00	2.83 ± 0.02	43.08 ± 0.08	14.2
	Control	0.45 ± 0.04	1.22 ± 0.03	36.98 ± 2.98	0.00
75	*T. versicolor*	0.13 ± 0.03	3.41 ± 0.05	41.50 ± 1.09	4.75
	B. adusta	0.27 ± 0.08	3.01 ± 0.07	39.28 ± 2.84	– 0.64
	P. chrysosporium	0.18 ± 0.05	2.86 ± 0.03	37.73 ± 1.10	– 4.80
	P. ostreatus	0.12 ± 0.00	2.91 ± 0.04	40.17 ± 1.10	1.59
	I. lateus	0.11 ± 0.02	2.70 ± 0.01	38.07 ± 0.32	– 3.84
	Control	0.26 ± 0.05	3.96 ± 0.02	39.53 ± 1.01	0.00
100	*T. versicolor*	0.16 ± 0.02	2.56 ± 0.04	33.72 ± 0.03	3.77
	B. adusta	0.26 ± 0.01	2.28 ± 0.01	35.16 ± 0.04	7.71
	P. chrysosporium	0.24 ± 0.00	2.17 ± 0.00	32.73 ± 0.01	0.86
	P. ostreatus	0.23 ± 0.03	2.27 ± 0.00	30.07 ± 0.11	– 7.91
	I. lateus	0.16 ± 0.00	2.21 ± 0.02	29.41 ± 0.04	– 10.3
	Control	0.45 ± 0.03	3.58 ± 0.01	32.45 ± 0.01	0.00