

BIODEGRADATION OF DISSOLVED HUMIC SUBSTANCES BY FUNGI

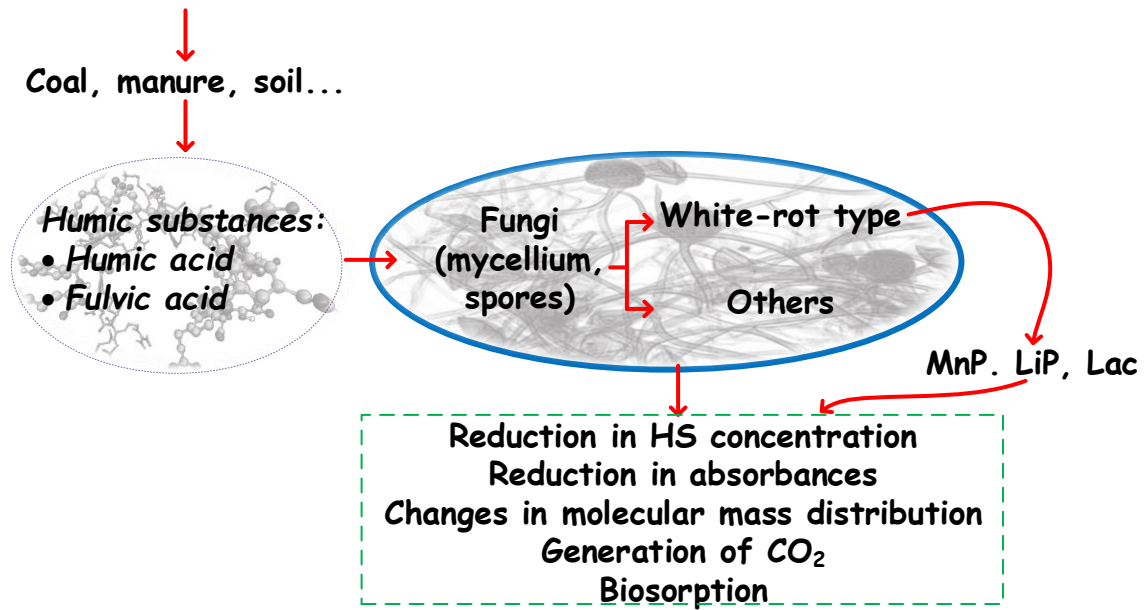
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Graphical abstract



Highlights

Importance of humic substances interaction with fungi

Fungal biodegradation is a complex function of the experimental conditions.

Enzymatic activity individually does not always fully explain the biodegradation.

Humic substances can be removed by biosorption as well.

Abstract

Humic and fulvic acids constitute humic substances, a complex mixture of many different acids containing carboxyl and phenolate groups, which are not only the principal soil fertility factors but also the main pollutants present in landfill leachates or natural organic matter in water. Due to their low bacterial biodegradability, fungal biodegradation processes are key for their removal. The present study compiles and comments all the available literature on decomposition of aqueous humic substances by fungi or by their extracellular enzymes alone, focusing on the influence of the reaction conditions. The biodegradation extent mainly depends on the characteristics and concentration of the humic compounds, the type of microorganisms selected, the inoculation mode, the C and N sources, the presence of certain chemicals in the medium, the availability of oxygen, the temperature and the pH.

Keywords:

Humic substances, humic acid, fulvic acid, fungi, ligninolytic enzymes

Introduction.

When organic matter from coal, manure, compost, soil or water is extracted with alkali, the soluble fraction generated consists of humic substances (HS), whereas the insoluble one contains products called humin and non-humic matter (McDonald et al., 2004). The HS are the most widespread and ubiquitous natural nonliving organic materials in terrestrial and aquatic environments and they represent the major fraction of soil organic matter (Granit et al. 2007). Properties of soil like water holding capacity, sequestration of nutrients and its transport, biological activity are directly affected by humic substances. If HS is subjected to a subsequent acid extraction, the solubilized polymers

are known as fulvic acids (FA), and those which remain in solid state correspond to humic acids (HA). Both recalcitrant acids are also the main components of natural organic matter in water (Bhatnagar and Sillanpää, 2017) and of old-landfill leachates (Gao et al., 2015), being occasionally used as model pollutants in laboratory tests for purifying drinking water (Kim and Dempsey, 2013) or for treating wastewater (Oulego et al., 2015, 2016). Nonetheless, since HA and FA contain lignin-like moieties such as anthraquinones, substituted benzoic acids and substituted cinnamic acids, they could also be employed as surrogates for wastewaters from textile, pulp and paper, olive mill and brewery industries, amongst others (Belcarz et al., 2005), which is another reason for their environmental importance.

In nature, humic substances are extremely resistant to biodegradation. It is now known that several microorganisms, mainly fungi, can decolorize and even completely mineralize humic acids under certain environmental conditions. Fungi are classified in four or five phyla, being those belonging to the Ascomycota and to the Basidiomycota the most frequent in the soil and employed for bioremediation (Sankaran et al., 2010; Tortella et al., 2005). Within both phyla, the wood-degrading type, and specially the white-, brown- and soft-rot fungi, deserve a special mention: they excrete extracellular enzymes that decompose complex nutrients into simpler substances that can be easily absorbed through the cell wall.

White rot and brown rot are mainly exerted by basidiomycetes, whereas soft rot is mainly performed by ascomycetes (Kües, 2015). Brown-rot fungi attack lignin by Fenton chemistry, turning it into a brown oxidized form and then, degrading it by means of the enzymes. White-rot fungi attack directly the lignin (or lignin-like) polymers through ligninolytic enzymes, mainly lignin peroxidase (LiP), manganese peroxidase

(MnP) and laccase (Lac) (Yadav and Yadav, 2015). Soft-rot fungi only perform a partial enzymatic degradation of cell wall polysaccharides and slight alterations of lignin. Lac [EC 1.10.3.2] are copper-containing oxidases that utilize molecular oxygen as oxidant, whereas peroxidases are heme proteins that catalyze the reduction of the hydrogen peroxide endogenously generated. The mechanism of LiP [EC 1.11.1.7] includes the oxidation of veratryl alcohol (an endogenously produced redox mediator) and the action of MnP [E.C.1.11.1.13] involves the oxidation of Mn(II) to Mn(III) prior to the oxidation of the targeted substrates (Ikehata et al., 2004). Other enzymes unable of breaking lignin, but implicated in the process, such as glycosyl oxidase and superoxide dismutase (both related with the generation of H₂O₂), glucose oxidase, cellulose-quinone oxidoreductase, aryl alcohol oxidase (AAO), horseradish-like peroxidase (HRP), cytochrome P450 enzymes and tyrosinase (also known as monophenol monooxygenase, MMO) are produced by the fungi as well (Pointing, 2001).

In this study, all the literature published to the date about application of fungi and their enzymes to bioremediation of HS-containing water is compiled and discussed. Unlike the existing review of Grinhut et al. (2007), the present one is not a description of the performance of these microorganisms in natural environments, but an analysis of the effect of the experimental variables on the fungal biodegradation of HA or FA in aqueous media,. Additionally, this paper also addresses the combination of biosorption with biodegradation, since fungi can also remove some compounds by adsorption to the biomass (Zhou and Banks, 1993).

General remarks

Precursor compounds of HS

The formation of humic substances is one of the least understood aspects of humus chemistry and one of the most intriguing. Preliminary understandings about how humic substances are formed is based on 4 published theories: lignin modification, quinone amino acid interaction, microbial synthesis of aromatics, and the Mallard reaction. Each theory describes complicated biotic and abiotic reactions in which a variety of organic compounds, such as phenolic compounds (eg. lignins), complex carbohydrates, and nitrogenous substances are resynthesized to form large complex polymers. The extreme variability in the molecular features of humic substances relates back to the precursor compounds and the environmental conditions under which the humic substances formed (Stevenson, 1994; Tan, 2014). Therefore, the parent material from which HS are formed is a variable taken into consideration.

In this regard, 41 references investigating fungal decomposition of HS, HA or FA in liquid media have been compiled. Table 1 presents them briefly. Regarding the origin of the HS employed, 16 of them are focused on compounds extracted from soil (Belcarz et al., 2005; Blondeau, 1989; Burges and Latter, 1960; Dehorter and Blondeau, 1992; Dehorter et al., 1992; Gramss et al., 1999; Koukol et al., 2004; Mathur, 1969, 1970; Mathur and Paul, 1966, 1967; Mishra and Srivastava, 1986; Paul and Mathur, 1967; Řezáčová et al., 2006; Yanagi et al., 2002, 2003), 13 on HS or HA from coal (Fakoussa and Frost, 1999; Hofrichter and Fritsche, 1997a; Kabe et al., 2005; Ralph and Catcheside, 1994, 1998a, b, 1999; Ralph et al., 1996; Temp et al., 1999; Willmann and Fakoussa, 1997; Wondrack et al., 1989; Zahmatkesh et al., 2016; Ziegenhagen and Hofrichter, 1998), 3 on HA from compost (Grinhut et al., 2011a, b; Kluczek-Turpeinen

et al., 2005) and 1 on HS, HA and FA from groundwater (Claus and Filip, 1998). Besides, 3 works use semi-synthetic or synthetic HA (Haider and Martin, 1988; Hofrichter et al., 1998; Wunderwald et al., 2000).

TABLE 1

There are also papers that utilize compounds from two origins: soil and coal (Kornilowicz-Kowalska et al., 2008; Zavarzina et al., 2004), soil and manure (Khandelwal and Gaur, 1980), soil and commercial (Petrovic et al., 1993) and litter and synthetic (Steffen et al., 2002). Of the 41 references, 1 deals with HA, HS and FA (Claus and Filip, 1998), 3 with HA and FA (Mathur, 1970; Petrovic et al., 1993; Řezáčová et al., 2006), 3 with HS (Gramss et al., 1999; Hofrichter and Fritsche, 1997a; Temp et al., 1999), 1 with FA (Mathur, 1969) and the remaining 33 with HA alone.

It has to be pointed out that the works of Hofrichter and Fritsche (1997a) and Temp et al. (1999) erroneously employed the term HA to designate HS, because the second acidic step was not performed, and that Belcarz et al. (2005) called HA to both true HA and to a mixture of HA and humin (therefore, this last mixture is not considered in this review).

We have not considered those publications that only studied degradation in solid media, such as those of Hurst et al. (1962), Hofrichter and Fritsche (1996, 1997b), Steffen et al. (2000), Elbeyli et al. (2006), Granit et al. (2007) and Šnajdr et al. (2010), nor the experiments in solid media of those articles working with liquids as well (Belcarz et al., 2005; Burges and Latter, 1960; Kluczek-Turpeinen et al., 2005; Kornilowicz-Kowalska et al., 2008; Řezáčová et al., 2006; Steffen et al., 2002; Zahmatkesh et al., 2016).

Measurement methods

The degradation of HS by fungi is mainly followed by ultraviolet-visible spectrophotometry. Most of the researchers measured absorbances at wavelengths between 275 and 665 nm, and estimated decolorization or bleaching through the decrease in these absorbances. However, a few authors stated that some wavelengths are better than others for evaluating certain properties, although they do not agree regarding to specific values. For example, Řezáčová et al. (2006) associated absorbances at 280 nm with the phenolic content or the aromaticity of the compounds, and absorbances at 665 nm with the color, whereas Kabe et al. (2005) affirmed that color is better determined at 480 nm and depolymerization at 275 nm. Belcarz et al. (2005) and Kornilowicz-Kowalska et al. (2008) used 400 – 450 nm for color and 500 nm for vanillic acid-like compounds (methoxyphenolic levels). Kluczek-Turpeinen et al. (2005) selected 450 nm for HA monitoring and 360 nm for FA monitoring without further explanations, despite the fact that the rest of literature indistinctly employed these wavelengths for both acids (Fakoussa and Frost, 1999; Hofrichter and Fritsche, 1997a; Řezáčová et al., 2006). Moreover, ratios of absorbances at two different wavelengths in the ultraviolet (Ralph and Catcheside, 1999) or visible ranges (Claus and Filip, 1998; Koukol et al., 2004; Mathur and Paul, 1967; Řezáčová et al., 2006; Zavarzina et al., 2004) are also employed to indicate the molecular weight evolution and the aromaticity degree (McDonald et al., 2004).

Other techniques used to quantify the HS biodegradation are the recovery and weighing of the remaining compounds (Burgess and Latter, 1960; Mathur and Paul, 1967), the changes in total organic carbon (TOC) (Řezáčová et al., 2006) or in chemical oxygen demand (COD) (Petrovic et al., 1993), and the measurement of the free fluorine (Wunderwald et al., 2000) or carbon dioxide (Haider and Martin, 1988; Hofrichter et al.,

1998; Steffen et al., 2002) generated during the mineralization of fluorinated or ^{14}C -labelled polymers, respectively.

Besides quantification, characterization of HS, HA and FA before and after the fungal treatment is usually performed as well, by means of techniques such as elemental analysis, Fourier transform ion cyclotron resonance mass spectrometry, nuclear magnetic resonance (of ^{13}C or of H) and size exclusion chromatography (largely the gel permeation type), being the last one the most utilized. Biodegradation can be accompanied by the increase, decrease or non-change in the modal mass (Gramss et al., 1999; Ralph et al., 1996), the presence of new low-molecular-weight peaks in the chromatograms (Grinhut et al., 2011a) and the reduction of phenolic levels, measured as vanillic acid (Belcarz et al., 2005; Kornilowicz-Kowalska et al., 2008). As one example, Fig. 1 illustrates the evolution of some of the chromatograms for coal HA. Fig. 1A shows a 168-h coal HA biodegradation by MnP from *Clitocybula dusenii*, showing a preferential attack on the high-molar-mass fractions and the formation of new peaks in the low-molar-mass range (Ziegenhagen and Hofrichter, 1998), whereas Fig. 1B reports a less selective attack and the no formation of new low-molecular-weight fragments during the 14-day degradation by *Phanerochaete chrysosporium* (Ralph and Catcheside, 1994).

FIGURE 1

It must be pointed out in Fig. 1B that the modal mass of the HA after 5 days of fungal biodegradation is higher than that at the beginning of the reaction, which suggests a certain degree of polymerization of the remaining HA. This repolymerization ability was observed in some ligninolytic enzymes (Hofrichter et al., 1998; Ikehata et al., 2004; Kulikova et al., 2013), and, depending on the experimental conditions, it can led to the

apparition of new peaks in the big-size range (Hofrichter and Fritsche, 1997a) and/ or to an increase in the modal mass, temporarily (Ralph and Catcheside, 1998a) or permanently (Ralph and Catcheside, 1998b; Temp et al., 1999; Zavarzina et al., 2004).

Other parameters measured during the fungal biotreatment of HSs are the fungal growth and the enzyme activity. Bleaching is usually accompanied by a rise of the biomass weight. Nevertheless a higher growth rate does not always imply a better biodegradation (Dehorter and Blondeau, 1992; Ralph et al., 1996; Řezáčová et al., 2006; Zahmatkesh et al., 2016).

Regarding enzyme activities, they are estimated through oxidation of certain substrates, such as 2,2'-azinobis(3-ethylbenzthiazoline-6-sulphonate) by Lac and MnP (Steffen et al., 2002; Willmann and Fakoussa, 1997), syringaldazine by Lac (Fakoussa and Frost, 1999), 2,6-dimethoxyphenol or vanillylacetone by MnP (Dehorter and Blondeau, 1992; Ralph and Catcheside, 1994), veratryl alcohol by LiP (Blondeau, 1989), 3,4-dihydroxyphenylalanine by MMO (Gramss et al., 1999), guaicol by HRP (Belcarz et al., 2005) and anisalcohol by AAO (Kabe et al., 2005). The corresponding enzyme activity is expressed in terms of activity units (U), which reflect the micromoles of substrate consumed per minute, although occasionally they are also calculated as the change in the absorbance of substrates per minute (Dehorter and Blondeau, 1992; Zavarzina et al., 2004). At this point, it should be mentioned that the enzyme activities measured in absence of HS, are higher than those obtained in their presence (Blondeau, 1989; Hofrichter and Fritsche, 1997a; Ralph and Catcheside, 1994, 1998b, 1999; Zavarzina et al., 2004) or lower (Dehorter and Blondeau, 1992; Kluczek-Turpeinen et al., 2005; Steffen et al., 2002; Temp et al., 1999; Willmann and Fakoussa, 1997). Fig. 2 displays

an example of these differences during the biodegradation of a coal HA by three different fungi.

FIGURE 2

Several authors have reported that the activity for a specific enzyme depends on the origin of the humic compounds (Belcarz et al., 2005; Claus and Filip, 1998; Kornilowicz-Kowalska et al., 2008) and their concentration (Claus and Filip, 1998; Willmann and Fakoussa, 1997; Zavarzina et al., 2004), the nutrients content (Ralph and Catcheside, 1994) and on the presence of other substances (Dehorter and Blondeau, 1992; Ralph and Catcheside, 1999; Steffen et al., 2002) in the reaction medium, together with the availability of oxygen (Claus and Filip, 1998; Ralph and Catcheside, 1998a; Ralph et al., 1996).

Fungal degradation

Since the majority of the compiled papers deals with several matters, for the sake of clarity we have divided them in six tables (see supplementary material), depending on the studied variable.

Effect of C and N

Table S1 groups the studies dealing with the effect of the presence of nutrients in the culture medium.

It can be seen in the works of Haider and Martin (1988) and Ralph and Catcheside (1994) (and of Blondeau (1989), Fakoussa and Frost (1999) and Grinhut et al. (2011b) if adsorption did not alter the general trends) that the higher the C/N ratio, the higher the biodegradation. However, this is not a general rule, because the sucrose removal in the

Czapek Dox broth fortified with yeast extract of Mathur (1970) did not worsen but enhanced the soil FA decolorization; HA degradation did not reach the highest percentage when the humic compound was the only source of nitrogen for the fungi of Mathur and Paul (1967), Khandelwal and Gaur (1980) and Mishra and Srivastava (1986); and two of the four fungi tested by Zahmatkesh et al.(2016) were more effective in the nitrogen supplemented medium than in the nitrogen limited one.

Taking into account that the absence of other sources of N and C led to equal or better performance than the presence of only C or only N for some microorganisms (Mathur and Paul, 1966, 1967; Mishra and Srivastava, 1986), it seems that the fungal decomposition of humic matter is a complex function of the N and C concentrations, and not only of the C/N ratio. Furthermore, if the data of Willman and Fakoussa (1997) and Fakoussa and Frost (1999) for adsorption plus biodegradation when HA was the only source of C or of N are extrapolable to biodegradation only, the fungal behavior turned out to be dependent on the nutrient source, and the results in different media will be not easily predictable (Gramss et al., 1999; Koukol et al., 2004; Mathur, 1969).

Effect of reaction medium and operational conditions

Table S2 includes those studies focused on the effect of the reaction medium and the operational conditions on the fungal degradation of HS.

Zahmetkesh et al. (2016), reported that the presence of an inhibitor of cytochrome P450 enzymes in the medium lowered the decolorization rate of the *Trametes versicolor*, thus probing that these enzymes were somehow implied in the decomposition of coal HA. Presence of Mn(II) stimulates the production of MnP (and of other enzymes at certain concentrations) and enhances the biodegradation (Steffen et al., 2002), although both,

the MnP activity and the bleaching, tend to a plateau with increasing Mn(II) concentrations (Ralph and Catcheside, 1998a). Besides, if adsorption does not play an important role, in the findings of Grinhut et al. (2011b), it has been shown that Mn(II) is more effective in some media than in others.

Addition of veratryl alcohol increased the activities of LiP and MnP without improving the bleaching in the paper of Ralph and Catcheside (1998b), possibly due to competition between the oxidations of the HA and the veratryl alcohol by the enzymes. Dehorter and Blondeau (1992) reported the same behaviour than Ralph and Catcheside (1998b), but, since they do not inform if adsorption was taken into account, this phenomenon could mask their results (in fact, the abrupt reduction of absorbance in the first days of incubation are common hints of biosorption processes) (Petrovic et al., 1993; Temp et al., 1999).

With regards to operational variables, aeration (as shaking) turn out to have a negative effect on the attack to FA by *Poria subacida* (Mathur, 1969), and to FA and HA by *Marasmius oreades* (Mathur, 1970), but a positive one for the decolorization of HA by *Penicillium frequentans* (Mathur and Paul, 1966, 1967) and *Cladosporium cladosporoides* (Claus and Filip, 1998). Shaking enhances the nutrient distribution and oxygen transfer, but also breaks the fungal mycelium, so, there will be an optimal agitation speed for each case (Díaz, 2012; Hadibarata et al., 2013; Senthilkumar et al., 2014). A higher availability of dissolved oxygen from air in shallow cultures was the explanation of Ralph et al. (1996) to the higher bleaching and lower increases in modal mass in 10-mL unshaken cultures when comparing it with 15-mL unshaken ones (both contained in 1-L conical flasks). On the other hand, Ralph and Catcheside (1998a) recommended the use of pure oxygen instead of air during the fungal biodegradation,

because the oxygenated media led to lower decolorization and to higher modal masses of the remaining HA.

Ralph and Catchside (1998a) were also the only ones that analyzed the influence of temperature in fungal behavior, observing that results at 37°C were better than at 28°C. They did not try to find the optimal temperature, that could be expected because the enzyme denaturalization and cell damage will increase at the higher temperatures.

Effect of the initial concentration

Table S3 summarizes the findings about the effects of the initial HS concentration on their fungal decomposition. Biodegradation rates of commercial HA by *Trichoderma viride* (Petrovic et al., 1993) and of soil FA by *Poria subacida* (Mathur, 1969) or *Trichoderma viride* (Petrovic et al., 1993) decreased when increasing the initial content of the humic material, whereas the attack to compost HA by *Pecilomyces inflatus* (Kluczek-Turpeinen et al., 2005) improved.

Removal of ¹⁴C-labelled HA by *Phanerochaete chrysosporium* (Haider and Martin, 1988) and of soil HA by *Trichoderma viride* (Petrovic et al., 1993) remained almost insensitive to the initial amount of HA in the feed, but the bleaching of coal HA by *Trametes versicolor* (Fakoussa and Frost, 1999) or by the unclassified strain RBS 1k (Willmann and Fakoussa, 1997) went through a maximum with respect to the initial concentration of the HS. Nonetheless, it should be stressed that the maxima in removal rates were only seen by researchers who did not detach adsorption from biodegradation. In all the cases, the media contained additional sources of C and N.

The binding of chlorinated substances from herbicides and insecticides to the humic matrix did not always hinder the mineralization of these compounds, and sometimes, their degradation rate was higher than if not binded (Haider and Martin, 1988).

Effect of the fungal species

Table S4 collects those papers (or sections of papers) which compared: the bleaching of several HAs by one fungus (Belcarz et al., 2005; Haider and Martin, 1988; Kornilowicz-Kowalska et al., 2008), of several HAs by several fungi (Yanagi et al., 2002), of one HA by several fungi or different strains of the same fungus (Burges and Latter, 1960; Dehorter et al., 1992; Fakoussa and Frost, 1999; Grinhut et al., 2011a; Kabe et al., 2005; Ralph and Catcheside, 1998b; Ralph et al., 1996; Yanagi et al., 2003) and of one HS or FA by two or more fungi (Gramss et al., 1999; Řezáčová et al., 2006; Temp et al., 1999).

At this point, it should be mentioned that, although some researchers do not take into account the HS biosorption by the fungus during the biodegradation process (Belcarz et al., 2005; Dehorter et al., 1992; Fakoussa and Frost, 1999; Grinhut et al., 2011a; Řezáčová et al., 2006; Yanagi et al., 2002), this phenomenon can be very important. For example, Gramms et al. (1999) noticed that the bleaching of soil HS by brown-rot fungi seemed higher than that of the white-rot ones if removal due to adsorption was not rested. Additionally, Gramms et al. (1999) also found that white-rot and brown-rot fungi were better than terricolous basidiomycetes, ectomycorrhizal fungi and soil-borne microfungi (with the exception of *S. lignicola*), whereas the brown-rot ones (*F. pinicola*, *P. betulinus*) could led to higher biodegradations than some white-rot microorganisms (*G. sapineus*, *K. mutabilis*, *P. ostreaus* and *S. rugoso-annulata*).

Miscellany

Table S5 included experiments which are not related with comparisons of nutrients, reaction media, operational conditions, HS concentrations or different fungi, and therefore, they could not be incorporated in the previous tables (Fakoussa and Frost, 1999; Mathur and Paul, 1966, 1967; Paul and Mathur, 1967; Willmann and Fakoussa, 1997; Wunderwald et al., 2000). It is worth stressing the results of Mathur and Paul (1966, 1967), Paul and Mathur (1967) and Willman and Fakoussa (1997), which reported an increase in the carboxylic groups and a decrease of the aromaticity of the HS after the fungal attack, either with or without adsorption of humic matter to the mycelium. This is in accordance with the studies of Claus and Filip (1998) and Grinhut et al. (2011a) and in opposition to the findings of Dehorter et al. (1992), cited in Tables S2 and S4. Taking into account that these three last works used the same microorganisms and medium, the behavior previously cited suggests an important biosorption in the work of Dehorter and Blondeau (1992), with a high attachment of the HA to the cells, stronger than in the works of Fakoussa and Frost (1999) and of Grinhut et al. (2011a).

Enzymatic degradation

Table S6 collects the experiments in the literature related to the bleaching of HS, HA or FA by fungal enzymes, in absence of the microorganism. The role of these enzymes in the biodegradation of humic compounds had semi-quantitatively been addressed by several authors (Tables S1 to S5), mainly studying if the increases in the enzymatic activity and in the HS decolorization are simultaneous. In this regard, some authors, such as Ralph and coworkers (Ralph and Catcheside, 1994, 1998a, b; Ralph et al., 1996), Kabe et al. (2005) and Zahmatkesh et al. (2016) preferred the comparison

between the decolorization degree with the activities measured in an HA-free medium, because it is alleged that HA interferes with assays for some enzymes, reducing their activities by formation of enzyme-HA complexes that do not oxidize the substrates (Ralph and Catcheside, 1994, 1998b; Wondrack et al., 1989).

In systems with one fungus and one or two enzymes, where agitation improved both biodegradation and enzyme activities (Claus and Filip, 1998), excretion of MnP and LiP in one medium was accompanied by bleaching, but the excretion of LiP alone in other medium caused not decolorization (Ralph and Catcheside, 1994). A strain that only produced MnP increased the modal mass of the remaining HA, but other strain, that generated both MnP and LiP, reduced it (Ralph and Catcheside, 1998b). When the parameters studied were temperature (28 or 37°C), veratryl alcohol (presence or absence) and atmosphere (oxygen or air), it seemed that the fungal attack was highly correlated with the enzyme activity (Ralph and Catcheside, 1998a). However, Kluczek-Turpeinen et al. (2005) noticed that during the degradation of compost HA by *P. inflatus*, only Lac was excreted, but that its activity decreased when increasing the HA concentration, whereas the bleaching followed the opposite trend. This suggests that lignolytic enzymes are necessary, but not sufficient, to accomplish the oxidation.

In articles where several fungi were tested, the correlation between bleaching and enzymatic activity became less evident, since the microorganisms did not produce all the enzymes nor their activities allowed an easy comparison (Gramss et al., 1999; Ralph et al., 1996; Temp et al., 1999) or, simply, because the highest enzymatic activities did not correspond to the highest decolorizations (Gramss et al., 1999; Kornilowicz-Kowalska et al., 2008; Zahmatkesh et al., 2016). With this in mind, Gramss et al. (1999) reported that brown-rot basidiomycetes (e.g., *F. pinicola*), which did not produce extracellular

phenol oxidases, decreased absorbance and altered the molecular weight of HS as effectively as white-rot basidiomycetes (e.g., *H. frowardii*), which produced all of these enzymes. The only exception is the work of Kabe et al. (2005), who compared three unidentified white-rot fungi, which generated four enzymes each one, and observed good correlation between decolorizations and depolymerizations with Lac and MnP activities.

When the HA removal due to adsorption was not taken into account, the majority of authors reported no correlation between both parameters (Dehorter and Blondeau, 1992; Fakoussa and Frost, 1999; Grinhut et al., 2011b; Řezáčová et al., 2006; Willmann and Fakoussa, 1997), although in some simple cases, the higher activities were linked to the higher bleachings (Belcarz et al., 2005; Blondeau, 1989; Steffen et al., 2002).

Considering these incongruences, Ralph and Catcheside (1998b), Wunderwald et al. (2000), Hofrichter and Fritsche (1997a) and Willman and Fakoussa (1997) tried to determine if MnP from fungi that were capable of bleaching humic compounds, could also decolorize the same substances in absence of the microorganisms. In the same way, this was the initial aim of Claus and Filip (1998) with Lac but they realized that the enzyme from the basidiomycete they had been working, displayed a very low activity, and preferred to utilize Lac from an ascomycete. Both Wunderwald et al. (2000) and Hofrichter and Fritsche (1997a) reported decreases in absorbance, but Willman and Fakoussa (1997) did not, a failure that these last ones tried to explain by the necessity of adsorption or proximity of the pollutants to the cell wall. Ralph and Catcheside (1998b) did not report absorbance reduction either, but noticed a rise in the modal mass, caused by the Mn(III) species, which also could cause a depolymerization if the atmosphere became more oxidant.

Gramss et al. (1999) compared three (MMO, Lac, HRP) of the four enzymes generated by the microorganisms they had tested (see Table S4) with other three enzymes and two abiotic oxidants (one of them contained Mn^{3+}). They also observed that HRP and the two abiotic oxidants gave the best HS removals, but since these researchers did not measure their activities, no useful information could be extracted from this comparison.

Other papers are focused on the optimization of the degradation process or on the better understanding of this process. Ziegenhagen and Hofrichter (1998) modified the amounts of HA, glutathione and organic solvents N,N-dimethylformamide and dimethylsulfoxide in order to improve the performance of MnP, Hofrichter et al. (1998) combined the same enzyme (but from other fungus) with several concentrations of the thiol mediator glutathione, Claus and Filip (1998) added 0 – 4 mmol/L of the redox mediator 1-hydroxybenzotriazole to Lac, and Zahmetkesh et al. (2016) compared four Lac mediators at 1 mmol/L. Wondrack et al. (1989) varied pH and LiP, HA, veratryl alcohol and H_2O_2 concentrations during the degradation of coal HA, but did not study all the combinations and left unspecified several parameters in their paper, which does not allow to know the optimum conditions. Also Ralph and Catcheside (1999) studied the effect of veratryl alcohol, $NaVO_3$ and oxygen sparging on the oxidation of coal HA and methylated coal HA by LiP from *P. chrysosporium*. The results of these last ones, and those of Zavarzina et al. (2004) with Lac from *P. tigrinus* confirmed that the enzymatic action depends on the HA characteristics, and that the same experimental conditions can lead to depolymerization in one acid and to polymerization in others.

Existence of metabolites

The generation of substances from 0.08 to 0.8 kDa observed in Fig. 1A is due to the fact that fungi or their enzymes do not always mineralize the HA or FA to CO₂ completely. Occasionally, the amount of these low- and medium-molecular-weight degradation products can be important, new peaks appear in the chromatograms and the mass distribution, which was initially unimodal, becomes bimodal (Hofrichter et al., 1998; Grinhut et al., 2011a) or multimodal (Wondrack et al., 1989). In the degradation of lignite HA with LiP from *Phanerochaete chrysosporium*, variation of the experimental conditions can avoid their formation (Wondrack et al., 1989).

Nonetheless, the absence of new prominent peaks does not imply the absence of metabolite generation. Mathur and Paul (1967) separated chromatographically soil HA in four fractions (higher than 35 kDa, between 8 and 35 kDa, between 6 and 8 kDa and lower than 6 kDa), finding that the lighter fraction was the most susceptible of suffering the attack by *Penicillium frequentans* (followed by the heaviest fraction). This suggested that, in the mixture of fractions, the larger molecules were not fully decomposed, but converted to intermediate and small-sized substances. By means of paper and gas chromatography, these authors found that two of these small-sized chemicals were salicyl alcohol and salicyl aldehyde (Mathur and Paul, 1966).

Gas chromatography was also employed by Ralph and Catcheside (1999) when dealing with the low-molecular-weight fraction from a methylated coal HA degraded by LiP from *Phanerochaete chrysosporium* in a medium with H₂O₂ and veratryl alcohol. They reported very small yields of fluoranthene, methyl 3,4-dimethoxybenzoate, *trans*-chrysanthenol, (*E*)-1-methylsophinyl-2-phenylethene, 2,5-dimethoxyethylbenzene, 4,6-dimethoxy-3(2*H*)-benzofuranone, 2,6-dimethoxytoluene, 4-methylbenzenecarbothioic acid *O*-methyl ester and methyl 3,4,5-trimethoxy-2-methylbenzoate.

With regard to intermediate-sized metabolites, Gramss et al (1999) reported that the forest mull HS attacked by *F. pinicola*, *L.deliciosus*, *H. crustuliniforme*, *S. lignicola*, *S. granulatus* and *P. involutus*, generated optically active substances, some of which resembled melanins. For the last two fungi, these metabolites also interfered with the colorimetric and chromatographic measurements. When the HS was treated with *H. frowardii* and *P. ostreatus*, there was a production of mono- and polysaccharides based on hexose sugars.

In the degraded lignite HSs used by Temp et al (1999) and Hofrichter and Fritsche (1997), a decrease in the HA fraction and an increase in the FA one were detected, indicating that the FAs were the intermediate-sized metabolites, since they were generated instead of removed by the fungal/ enzymatic attack. When Fakoussa and Frost (1999), Kluczek-Turpeinen et al. (2005), Steffen et al. (2002), Wunderwald et al. (2000), Zahmatkesh et al. (2016) and Ziegenhagen and Hofrichter (1998) subjected their degraded HAs to an acid extraction, true FAs were also found in the supernatants.

Comparison with other actual pollutants

Bioremediation of complex effluents by fungi has generated a huge amount of literature. This has been partially compiled by Singh (2006), and there are also several specific reviews on textile (Bilal et al., 2017; Fu and Viraraghavan, 2001; Holkar et al., 2016; Rodriguez-Couto, 2009; Sen et al., 2016; Wesenberg et al., 2003), pulp and paper (Bajpai, 2012; Kamali and Khodaparast, 2015; Rubilar et al., 2008), olive mill (Mann, 2011; McNamara et al., 2008; Morillo et al., 2009) and brewery wastewaters (Pant and Adholeya, 2007; Satyawali and Balakrishnan, 2008). However, to the best of our

knowledge, only one of the studies cited in those papers compares the degradation of one industrial pollutant with that of HA: the work of Belcarz et al (2005).

The main results reported by these authors were summarized in Table S4. They also tested the biodegradation of a daunomycin post-production stream with *Bjerkandera adusta* R59 in a liquid Park and Robinson medium with 10% wastewater (total daunomycin content of 8.9 mg/L and phenolic levels of 11 mg/L) at 26°C for 21 days. Decolorization (59% at 7 days and 94% beyond 14 days) was higher than that of HAs from lessive soil or from chernozem in a Fahreus medium, but phenolic levels were higher (4.86 mg/L at the end of the experiment) and the peroxidase activity went through a maximum at 7 days instead of at 14 days.

Conclusions and future gaps

HS can be considered as model compounds of several types of pollutants present in wastewaters. Fungi (and especially those from the white-rot type) attack and decompose HA and FA mainly through extracellular ligninolytic enzymes (Lac, MnP and LiP). The effectiveness of the biodegradation has shown to be a complex function of reaction time, type of fungus, inoculation mode, type of C and N sources and their concentrations, origin, composition and concentration of the HS, presence of promoters or inhibitors of the fungal growth or of fungal activity, pH, temperature and aeration, and therefore, they have to be optimized for each individual HS and fungus. Additionally, the cells can remove part of the HS by means of adsorption, and this should be taken into account for either the use or disposal of the excess biomass.

The use of ligninolytic enzymes has proven to be an alternative to the utilization and characterization of fungi, since their activity in the liquid medium does not depend on nutrients and fungus metabolism. However, the costs associated to their production and separation reduces the competitiveness of this option when compared to the biomass-based processes.

However, there is much work remaining to be done. For instance, the effects of variables related to the scale-up of the biotreatment, such as the fungal immobilization or the interactions between fungi and bacteria (for bioaugmentation processes or during the treatment of NOM or leachates, for example) have not yet been studied. There is a lack on in-depth knowledge about the characterization/quantification of the intermediates and products generated during the biodegradation. Additionally, their separation and recovery have not been considered. In a similar way, the effects of fungal treatment on the subsequent discharge or downstream processing of the effluent are unknown.

Fig. 3 shows a scheme of the different variables with effect on the biodegradation of humic and fulvic acids by fungi, giving an idea of those gaps that will need more research.

FIGURE 3

Acknowledgements

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Supplementary data

Tables S1- S6

Ethical Standards

Sergio Collado declares that he has no conflict of interest.

Paula Oulego declares that she has no conflict of interest.

Octavio Suárez-Iglesias declares that he has no conflict of interest.

Mario Díaz declares that he has no conflict of interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

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Figure 1

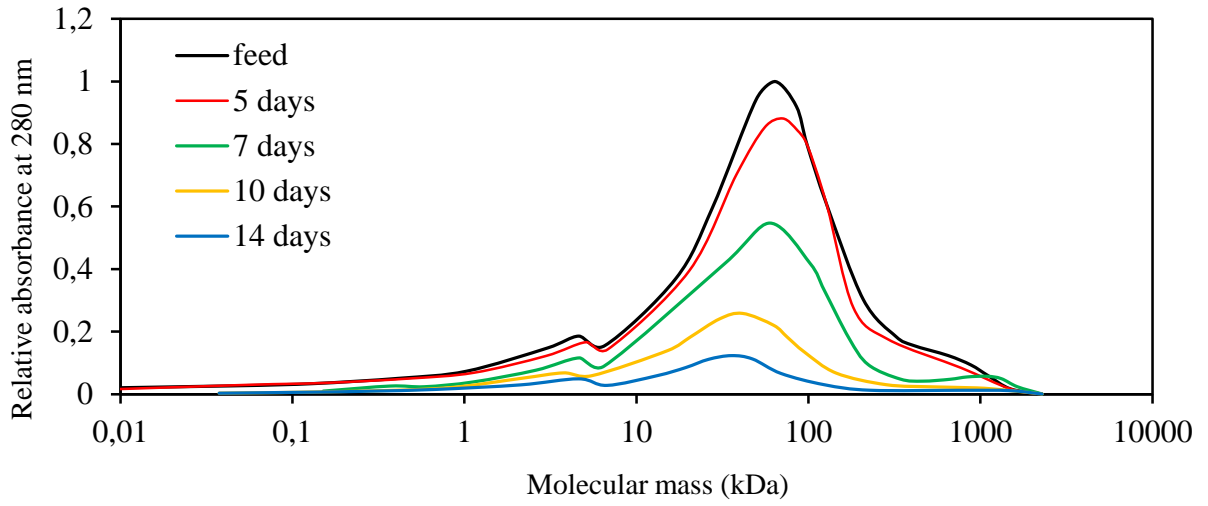
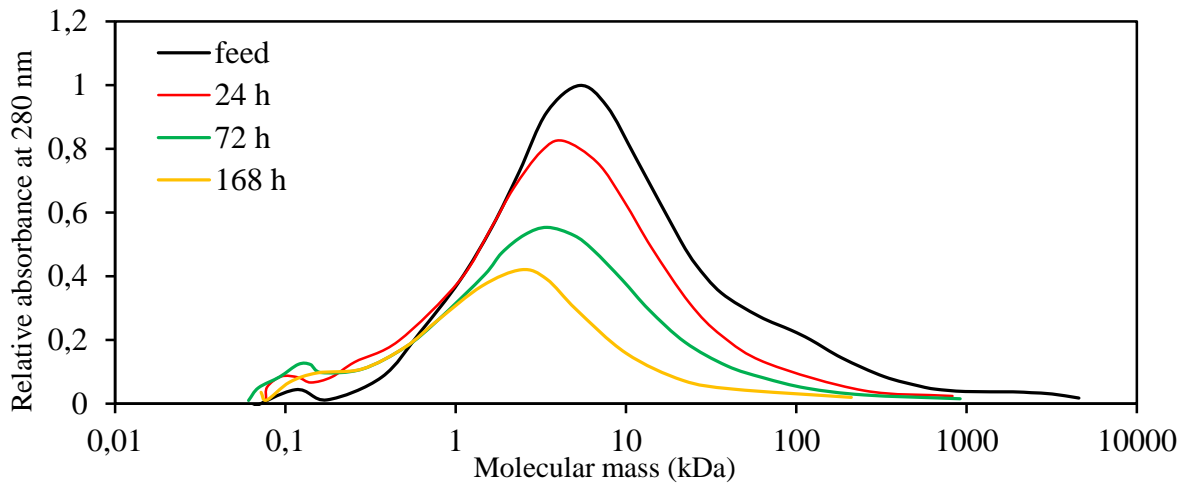


Figure 2

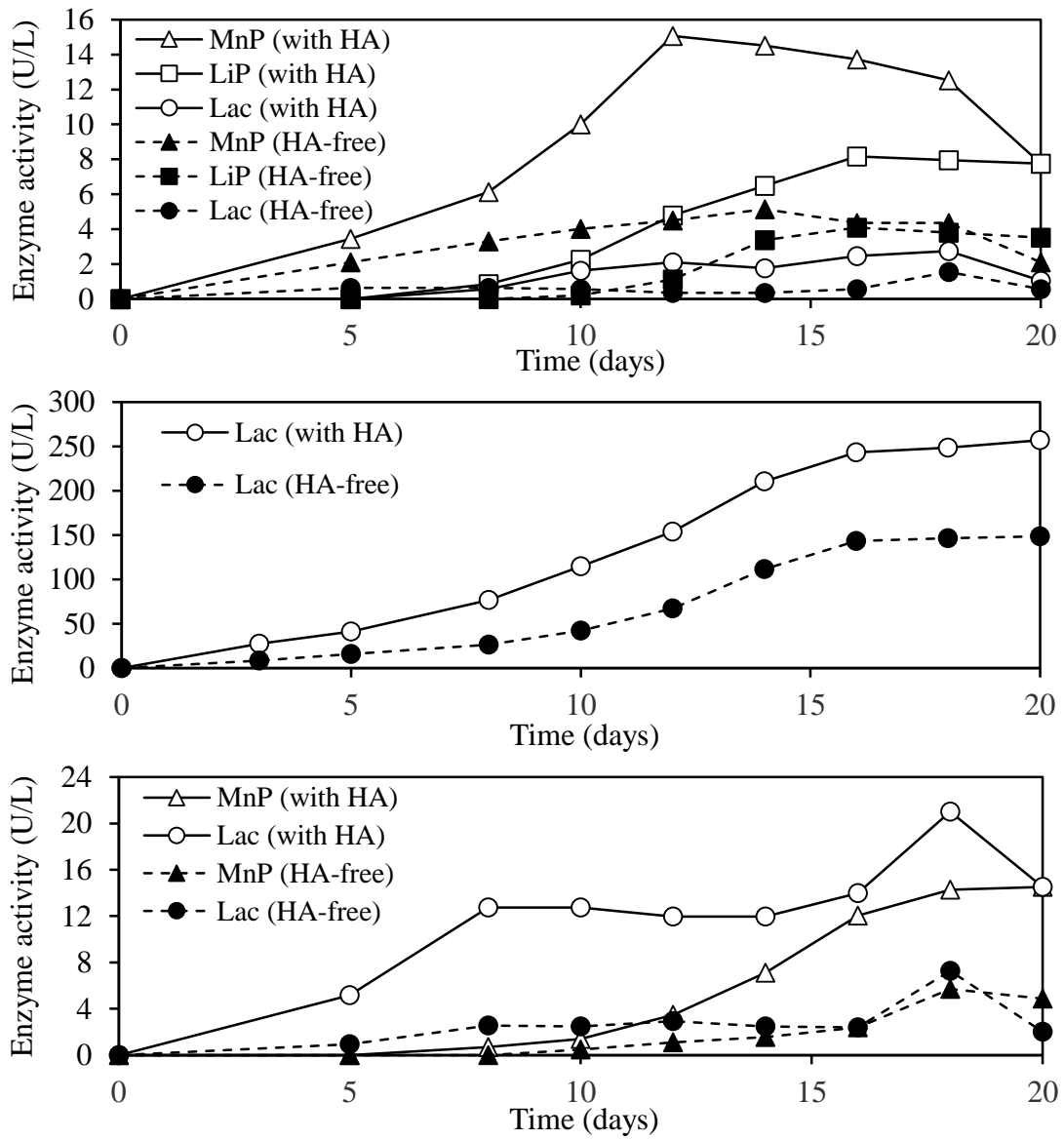


Figure 3

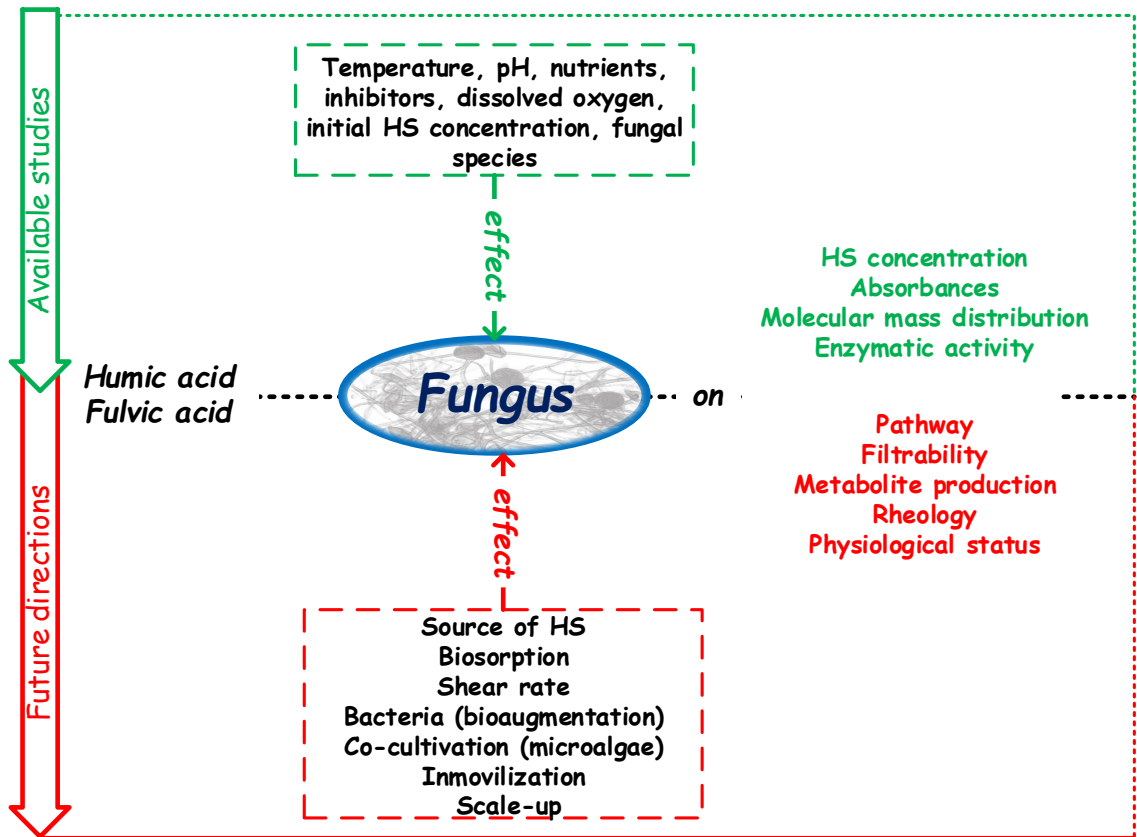


Figure captions

Fig. 1. Time-evolution of the molecular mass distribution for a) coal HA attacked by MnP from *Clitocybula duseinii* according to Ziegenhagen and Hofrichter (1998) and for b) coal HA degraded by *Phanerochaete chrysosporium* according to Ralph and Catcheside (1994). For further information, see Tables S6 and S1, respectively.

Fig. 2. Variation of the enzymatic activities with time in media with and without HS for a) basidiomycete JF 596, b) *Pycnoporus cinnabarinus* and c) *Polyporus ciliatus* according to Temp et al. (1999). For further details, see Table S4.

Figure 3.- Summary of the available studies and the future directions on the identified knowledge gaps related to the fungal biotreatment of humic substances.

Supplementary material for

BIODEGRADATION OF DISSOLVED HUMIC SUBSTANCES BY FUNGI. A REVIEW

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Table S2. Influence of reaction medium and operational conditions in the fungal decolorization of humic compounds.

Table S3. Influence of feed concentration in the fungal decolorization of humic compounds.

Table S4. Comparison of several fungi or of humic compounds under the same experimental conditions for the bleaching.

Table S5. Operational features of some fungal decolorizations, not previously cited.

Table S6. Decolorization of humic compounds by means of fungal enzymes alone.

Table S1

Influence of nutrients in the fungal decolorization of humic compounds.

Autor	Fungi, medium and experimental conditions	Comments and results
Mathur and Paul (1967)	<ul style="list-style-type: none"> • HA from the Ap horizon of Melfort Orthic Black soil. • Seven fungi (two <i>Penicillium frequentans</i>, <i>Aspergillus versicolor</i>, <i>Fusarium</i> sp, <i>Cephalosporium</i> sp., <i>Cunninghamella</i> sp. and <i>Penicillium</i> 895) isolated from that soil. • 0.2% HA in a Czapek Dox broth without sucrose, nitrate or both, inoculated with 48-h old washed cells and shook at room temperature for 4 weeks. 	<ul style="list-style-type: none"> • Prior to measurements, cells were separated and washed with a buffer to recover the HA adsorbed by them. Washing was added to the solution. • When HA was supplied as the sole source of C, decolorizations (decreases of absorbance at 465 nm) were 19.2% for one <i>P. frequentans</i>, 3.8% for the other <i>P. frequentans</i> and for <i>Fusarium</i> sp., 9.6% for <i>A. versicolor</i>, and 7.7% for <i>Cephalosporium</i> sp., <i>Cunninghamella</i> sp. and <i>Penicillium</i> 895. When the acid was the sole source of N, bleachings were 17.3% for one <i>P. frequentans</i>, 3.8% for the other <i>P. frequentans</i>, -11.5% for <i>A. versicolor</i>, 5.7% for <i>Fusarium</i> sp., <i>Cephalosporium</i> sp. and <i>Penicillium</i> 895, and 7.7% for <i>Cunninghamella</i> sp. When HA was the sole source of both C+N, degradations were 17.3% for one <i>P. frequentans</i>, 7.7% for the other <i>P. frequentans</i>, for <i>Cephalosporium</i> sp. and for <i>Cunninghamella</i> sp., 5.7% for <i>A. versicolor</i> and <i>Fusarium</i> sp., and 11.5% for <i>Penicillium</i> 895.
Mathur and Paul (1966, 1967)	<ul style="list-style-type: none"> • HA from the Ap horizon of Melfort Orthic Black soil, with 54% C, 4.76% H, 3.89% N and 37.4% O (ash-free) • <i>Penicillium frequentans</i> from the same soil • 0.02% HA in a Czapek Dox broth without sucrose, nitrate or both. Inoculation with mycelium or spores and incubation for 7 weeks at room temperature with or without shaking. 	<ul style="list-style-type: none"> • Prior to measurements, cells were separated and washed with a buffer to recover the HA adsorbed by them. Washings was added to the cultures. • In the stationary culture inoculated with mycelium, the HA mass decreased a 10.2% when HA was the source of C and a 6.2% when it was the source of N. No measurements were done when it was the source of both C and N. • In the shaken culture inoculated with spores, the absorbance at 465 nm decreased a 6.6% when HA was the source of C, a 5% when it was the source of N and a 6.6% when it was the source of both C and N. • In the shaken culture inoculated with mycelium, the absorbance at 465 nm decreased a 31.6% when HA was the source of C, a 30% when it was the source of N and a 35% when it was the source of both C and N.

Autor	Fungi, medium and experimental conditions	Comments and results
Mathur (1969)	<ul style="list-style-type: none"> • FA from the Bh horizon of Armadale soil • The white-rot fungus <i>Poria subacida</i> 17780 • 200-mL Erlenmeyer flasks containing 25 mL Czapek-Dox broth fortified with 0.02% yeast extract at pH 5.0, with 0.05% FA, inoculated with 72-h cultures and incubated at 28°C without shaking for 24 days. • 300-mL Erlenmeyer flasks containing Czapek-Dox broth without sugar at pH 5, 0.05% FA as the sole source of carbon and inoculated with fungal pellets. Incubation at 28°C without shaking for 48 h 	<ul style="list-style-type: none"> • Solutions with FA adsorbed on mycelia served as controls in color comparisons. Pellets were presoaked in FA to avoid adsorption during the degradation. At the end of the experiments, all the FA on the cells was recovered by washing with 1% K₃PO₄. • In the Czapek-Dox broth fortified with yeast extract, decolorizations (reduction of absorbances at 465 nm) were of 3.8% after 10 days, 25.38% after 18 days and 45.3% after 24 days. • In the Czapek-Dox broth without sugar, decolorization was of 66.7% after 48 h
Mathur (1970)	<ul style="list-style-type: none"> • HA from chernozem and FA from podzol • Basidiomycete <i>Marasmius oreades</i>, also called the fairy ring mushroom. • 200-mL flask with 25 mL of Czapek Dox broth, 0.02% yeast extract and 460 ppm HA or 470 ppm FA, inoculated with one loopful (1-mm size) of homogenized culture and incubated at 24°C for 4 weeks without shaking. • 200-mL flask containing 25 mL of Czapek Dox broth without sugar but with 0.02% yeast extract and 468 ppm HA or 475 ppm FA, inoculated with 0.6 g wet cells and incubated at 24°C for 1 week without shaking 	<ul style="list-style-type: none"> • For eliminating the effect of fungal absorption, controls were carried out with two sets of flasks: one containing medium + FA or HA but let uninoculated and the other inoculated but without FA or HA. Concentrations were determined by absorbances at 465 nm. • In the Czapek Dox medium with yeast extract, the concentrations of HA and FA decreased a 22% and a 39%, respectively, after 4 weeks. • In the medium without sucrose, the concentration of HA decreased a 15% after 4 h and 18% after 1 week, whereas the FA content decreased a 44% at 4 h and a 51% at 1 week.

Autor	Fungi, medium and experimental conditions	Comments and results
Khandelwal and Gaur (1980)	<ul style="list-style-type: none"> • HA from alluvial soil and farmyard manure • <i>Fusarium solani</i> (F 5), <i>Penicillium roseopurpureum</i> (F 5) and <i>Aspergillus fumigatus</i> (F 7) • Broth with HA as the sole source of either carbon, nitrogen or both and also in the presence of glucose (0.25%) and ammonium sulphate (0.1%), inoculated with spore suspensions from 5-day-old fungal and 14-day-old bacterial cultures and incubated for 40 days. 	<ul style="list-style-type: none"> • Prior to measurements, cells were separated and washed with NaOH to recover the HA adsorbed by them. Washing was added to the solutions. • When the manure HA was supplied as the sole source of C, decolorizations (% reduction of absorbance at 430 nm) were 13.3% for <i>F. solani</i>, 12.2% for <i>P. roseopurpureum</i> and 24.4% for <i>A. fumigatus</i>. When the same HA was supplied as the sole source of N, they were 22.5% for <i>F. solani</i>, 17.8% for <i>P. roseopurpureum</i> and 15.6% for <i>A. fumigatus</i>. If HA was the sole source of both C and N, bleachings were 9.2% for <i>F. solani</i>, 10.5% for <i>P. roseopurpureum</i> and 9.2% for <i>A. fumigatus</i>. When both C and N sources were present, results were 40.0% for <i>F. solani</i>, 24.4% for <i>P. roseopurpureum</i> and 31.1% for <i>A. fumigatus</i>. • For the soil HA, decolorization percentage was only given for <i>F. solani</i>. It was 0.0% when HA was supplied as the sole source of C, N or C+N. When there were C and N sources, decolorization was of 18%.
Mishra and Srivastava (1986)	<ul style="list-style-type: none"> • HA from an Alfisol • <i>Aspergillus</i> sp. from Bangalore, Ranchi and Calcuta, <i>Aspergillus awamori</i>, <i>Humicola insolens</i>, <i>Paecilomyces fuisproms</i>, <i>Penicillium</i> sp, <i>Trichurus spiralis</i> and <i>Trichoderma viride</i>. • 250-mL conical flasks containing 50 ml of Czapek-Dox medium with 0.1% HA (w/v), but excluding (i) sucrose, (ii) nitrate or (iii) nitrate and sucrose, in order to fix HA as the sole source of (i) carbon, (ii) nitrogen and (iii) carbon and nitrogen. Inoculation with 10 mL suspension in sterile water of a 72 hour old culture grown on Czapeck-Dox agar slope, containing 19×10^4/mL viable cells. Incubation at 30°C for 6 weeks 	<ul style="list-style-type: none"> • Percentages of decolorization were measured as in Mathur and Paul (1967). • When HA was the sole source of C, they were 8.9% for <i>Aspergillus</i> sp. from Bangalore, 11.6% for <i>Aspergillus</i> sp. from Calcuta and for <i>Aspergillus</i> sp. from Ranchi, 30.2% for <i>A. awamori</i>, 25.0% for <i>H. insolens</i>, 14.6% for <i>P. fuisproms</i>, 27.6% for <i>Penicillium</i> sp., 22.4% for <i>T. spiralis</i> and 19.8% for <i>T. viride</i>. When HA was the sole source of N, they were 3.0% for <i>Aspergillus</i> sp. from Bangalore and <i>Aspergillus</i> sp. from Calcuta, 19.8% for <i>A. awamori</i>, 0.0% for <i>Aspergillus</i> sp. from Ranchi, 17.2% for <i>H. insolens</i>, 0.3% for <i>P. fuisproms</i>, 22.4% for <i>Penicillium</i> sp., 6.0% for <i>T. spiralis</i> and 11.6% for <i>T. viride</i>. When HA was the sole source of both C and N, they were 11.2% for <i>Aspergillus</i> sp. from Bangalore, 11.6% for <i>Aspergillus</i> sp. from Calcuta, 27.6% for <i>A. awamori</i>, 0.9% for <i>Aspergillus</i> sp. from Ranchi, 22.4% for <i>H. insolens</i>, 17.2% for <i>P. fuisproms</i> and <i>T. viride</i>, and 25.0% for <i>Penicillium</i> sp. and <i>T. spiralis</i>.

Autor	Fungi, medium and experimental conditions	Comments and results
Haider and Martin (1988)	<ul style="list-style-type: none"> • ¹⁴C-labelled HA from an Alfisol previously mixed with ¹⁴C-wheat straw. • <i>Phanerochaete chrysosporium</i> ATCC 32629 • 200-mL Erlenmeyer flasks with 50 mL liquid medium, containing the mineral salts and vitamins of Kirk et al. (1978), 0.56 mmol/L D-glucose as carbon source, variable amounts of NH₄NO₃ and L-asparagine as nitrogen sources and variable amount of HA, inoculated with spores and held at 35°C for 18 days. 	<ul style="list-style-type: none"> • Three concentrations of HA (100, 200 and 400 mg/L) were combined with three concentrations of nitrogen (0.6 mmol/L NH₄NO₃ + 0.6 mmol/L L-asparagine, 3 mmol/L NH₄NO₃ + 3 mmol/L L-asparagine and 6 mmol/L NH₄NO₃ + 6 mmol/L L-asparagine), obtaining a total number of nine experimental conditions. • The percentage of HA converted into ¹⁴CO₂ increased with the incubation time, but it was not considerably affected by the initial concentration of HA, only by that of the nitrogen source. After 18 days it was of 33 – 35% for 0.6 mmol/L of each nitrogenous compound, 18 – 19% for 3 mmol/L and 15 – 17% for 6 mmol/L.
Haider and Martin (1988)	<ul style="list-style-type: none"> • HA-like synthetic polymers prepared from a mixture of several substances with one ¹⁴C-labelled compound (catechol, 4-chlorocatechol, 4,5-dichlorocatechol, ferulate, aniline, 4-chloroaniline or protein hydrolysate) • <i>Phanerochaete chrysosporium</i> ATCC 32629 • 200-mL Erlenmeyer flasks with 50 mL liquid medium, containing the mineral salts and vitamins of Kirk et al. (1978), 0.56 mmol/L D-glucose as carbon source, 0.6 mmol/L NH₄NO₃ and 0.6 mmol/L L-asparagine as nitrogen sources (or the double, 1.2 mmol/L of each nitrogenous compound) and 100 mg/L HA, inoculated with spores and held at 35°C for 18 days. 	<ul style="list-style-type: none"> • The chlorinated aromatic moieties were biodegradation intermediates of herbicides and insecticides, which were known to be immobilized in soil by binding to the humus matrix. The main objective of the experiment was to determine if the fungus was able of mineralizing them as well as the non-chlorinated ones, which were known to be units of the HA. • The percentage of HA converted into ¹⁴CO₂ after 18 days in a medium of 0.6 mmol/L NH₄NO₃ and 0.6 mmol/L L-asparagine was of 55.9% for catechol, 38% for 4-chlorocatechol, 45.7% for 4,5-dichlorocatechol, 54.0% for ferulate, 17.5% for aniline, 2.4% for 4-chloroaniline and 24.7% for protein hydrolysate. • If the nitrogen source concentration was doubled, the mineralization was inhibited during the first 5 to 10 days, but after 18 days, the results were very close to those with 0.6 mmol/L of each nitrogenous compound.

Autor	Fungi, medium and experimental conditions	Comments and results
Blondeau (1989)	<ul style="list-style-type: none"> • Natural HA, extracted from a forest soil, with a modal mass of 23 kDa. • White-rot fungus <i>Phanerochaete chrysosporium</i>, strains DSM 1556 and BKM-F 1767 • Aerobic batch cultures in serum flasks of 500 mL with cotton stoppers. Each flask contained 50 mL of a sterilized aqueous medium which consisted of 0.05% HA, 2 mg/L thiamine, 0.6 mmol/L NH₄NO₃ and 0.6 mmol/L L-asparagine, buffered to pH 4.5 with 20 mmol/L sodium tartrate. 56 mmol/L glucose were incorporated in some assays. Inoculation was performed with conidial suspensions to obtain 20×10⁶ spores per flask. Cultures were incubated at 35°C for 18 days. 	<ul style="list-style-type: none"> • Without glucose, fungal growth (assessed by the weight of mycelium after drying at 105°C) and decolorization (evaluated by absorbance at 350 nm) were not seen. • With glucose, the growth of the two strains was similar (40 mg at day 2, 80 mg at day 4, 110 mg at day 9 and 165 mg at days 16 -18), but decolorization was only seen with the strain BKM-F 1767 (2% at day 4, 15% at day 4, 57% at day 14 and 61% at day 18). In this regard, no LiP activity of the DSM 1556 strain was observed, whereas the BKM-F 1767 one exhibited significant LiP activity. • LiP activity of the strain BKM-F 1767 was also measured in a medium without HA, finding that it was higher than that in the medium with HA. • None is said about the HA adsorbed by the fungal hyphae. • Gel filtration chromatography profiles showed a reduction of HA molecular weight after incubation for 15 days with strain BKM-F 1767. The modal mass was reduced to 12 kDa. The size changes were particularly evident in the high-molecular weight range (50 – 500 kDa). However, no accumulation of low-molecular-weight compounds was noticed

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Ralph and Catcheside, (1994)	<ul style="list-style-type: none"> • HA from Morwell brown coal • The white-rot fungus <i>Phanerochaete chrysosporium</i> strain BKM-F 1767 • 100-mL Erlenmeyer flasks with 10 mL low nitrogen or nitrogen sufficient medium, 0.03% HA (w/v), inoculated with 10⁵ conidia, closed with polystyrene foam bungs and incubated at 37°C in a water-saturated atmosphere without agitation for 16 days. Low nitrogen medium was that described by Tien and Kirk (1988), but was supplemented with 0.05% (w/v) oleic acid, 0.005% (w/v) Tween 80 and contained 2 mmol/L veratryl alcohol. Nitrogen sufficient medium was similar to low nitrogen medium except that it contained 52 mmol/L nitrogen as D-diammonium tartrate and no veratryl alcohol. Low nitrogen medium contained 2.17 mmol/L nitrogen as D-diammonium tartrate and 337 mmol/L carbon as glucose and tartrate. Nitrogen sufficient medium contained 437 mmol/L carbon as glucose and tartrate. 	<ul style="list-style-type: none"> • Reduction of the absorbance at 400 nm was of 40% at day 4 in the nitrogen sufficient medium and of 84% at day 5 in the low nitrogen medium. Thereafter, and within the experimental error, there was not considerable decolorization. Recovery of the adsorbed HA probed that all the bleaching in the nitrogen sufficient medium was due to bisorption, but that this phenomenon was negligible in the low nitrogen medium. • Gel permeation chromatography for the low nitrogen medium indicated that the modal mass slightly increased from 65 to 70 kDa in the first day of incubation, remained roughly constant until day 5 and then decreased, reaching a value of 32 kDa at day 16 (See Figure 1b). For the sufficient nitrogen medium, the modal mass increased from 65 to 76 between days 0 and 4, remained constant until day 8, then decreased to 61 kDa at day 12 and increased to 67 kDa at day 16, but these changes were attributed to experimental error. • Cell dry weight went through a maximum at 3-4 days and tended to a plateau at 16 days. Maxima were of 47 mg in the sufficient nitrogen medium and of 21 mg in the low nitrogen medium. Plateaux were of 23.5 mg in the nitrogen sufficient medium and of 16 mg in the low nitrogen medium. • Glucose was consumed faster in the nitrogen sufficient medium than in the low nitrogen medium. • LiP activity in the low nitrogen medium was 0 on day 5, peaked at 83 U/L on day 12 and reached a value around 30 U/L after 15 days. LiP activity was not detected in nitrogen sufficient medium. MnP activities were zero below 2 days and above 14 days, but went through maxima of 140 U/L at 4 days in the low nitrogen medium and of 95 U/L at 12 days in the sufficient nitrogen medium. Comparison with enzyme activities in the same media without HA revealed that HA considerably inhibited the activities of both LiP and MnP, and that in the low nitrogen medium, it changed the time at which the maximum of LiP appeared.

Autor	Fungi, medium and experimental conditions	Comments and results
Willmann and Fakoussa (1997)	<ul style="list-style-type: none"> • HA from german lignite, with 40.3% C, 4% H, 5.1% N, 24.9% O and 25% ash. • Fungal strain RBS 1k, a haploid basidiomycete isolated from an open-cast mining region (Cologne), probably belonging to the <i>Aphyllphorales</i> or <i>Agaricales</i> • 250-mL conical flasks with 80 mL medium and an unknown amount of HA, adjusted to pH 4.3, inoculated with a piece of fungal mycelium from malt-extract agar (0.5×0.5 cm) and incubated without shaking for 25 days. The basal solution contained 200 mg/L KH₂PO₄, 50 mg/L MgSO₄·7H₂O, 10 mg/L CaCl₂, 20 mmol/L carbon source, 220 mg/L ammonium tartrate, 1 mg/L thiamine, 1.46 g/L sodium dimethylsuccinate, 0.5 g/L Tween 80; 5 mL trace elements and 0.5 mL vitamin solution. The composition of the trace elements was 3.0 g/L MgSO₄·7H₂O, 0.5 g/L MnSO₄·H₂O, 1.0 g/L NaCl, 100 mg/L FeSO₄·7H₂O, 100 mg/L CoSO₄, 82 mg/L CaCl₂, 100 mg/L ZnCl₂, 10 mg/L CuSO₄·5H₂O, 10 mg/L AlCl₃, 10 mg/L H₃BO₃ and 10 mg/L NaMoO₄, whereas the vitamin solution consisted of 2 mg/L biotin, 2 mg/L folic acid, 5 mg/L thiamine hydrochloride, 5 mg/L riboflavin, 10 mg/L pyridoxine hydrochloride, 0.1 mg/L cyanocobalamine, 5 mg/L nicotinic acid, 5 mg/L calcium DL-pantothenate and 5 mg/L <i>p</i>-aminobenzoic acid. 	<ul style="list-style-type: none"> • In the mineral salt medium, the carbon sources were galactose, acetate, glutamate, glucose and glycerine. For the two last ones, decolorizations (as decrease of absorbances at 420 nm) were of 82%, whereas they were around 10% for the three first ones. • In principle, these bleachings include both biodegradation and adsorption. The authors affirmed that the mycelia became dark-coloured at the beginning of the experiment, but that after a few days, they were bright again. However, it is not clear if this statement refers only to the media with glucose and glycerine, or to all.

Autor	Fungi, medium and experimental conditions	Comments and results
Fakoussa and Frost (1999)	<ul style="list-style-type: none"> • HA from lignite • <i>Trametes versicolor</i> DSM 3086 • Presumably, standing flask cultures with malt extract medium diluted to 1/5 and an unspecified HA concentration around 0.5 – 1.2 g/L HA in an incubator at 30°C and an atmosphere of 40 vol% oxygen for 7 days. The C/N ratio of the medium (68) was varied between 46 and 76 with dextrin and ammonium nitrate. 	<ul style="list-style-type: none"> • Decolorizations at C/N ratios of 46, 68, 72 and 76 were 41, 50, 53.1 and 53.4%, respectively. These values include both biosorption and biodegradation. • Activities of the dominating enzyme (Lac) were measured: at C/N ratios of 46, 68, 72 and 76 were 640, 425, 360 and 339 U/L, respectively. It is not specified if these activities were averaged or those at the maximum.
Fakoussa and Frost (1999)	<ul style="list-style-type: none"> • HA from lignite • <i>Trametes versicolor</i> DSM 3086 • Standing cultures in Petri dishes with 25 mL medium and 0.05% HA in an incubator at 30°C, 90% relative humidity and 40 vol% oxygen. Three media were compared: diluted malt extract, mineral salts and peptone. The mineral salt medium was supplied with different sources of C (glucose, galactose, glycerol, dextrin and sucrose) and N (sodium nitrate, ammonium nitrate and ammonium sulfate). 	<ul style="list-style-type: none"> • Decolorization in the mineral salt medium followed the decreasing order: glycerol > sucrose > glucose > (NH₄)₂SO₄ > NH₄NO₃ > dextrin > NaNO₃ ≥ galactose. • Decolorizations in diluted malt and in peptone were the same than in the mineral salt medium with glycerol and dextrin, respectively. • Decolorization in yeast extract was between that in peptone and that in the mineral medium with NH₄NO₃ • These bleedings include both adsorption and biodegradation.

Autor	Fungi, medium and experimental conditions	Comments and results
Gramss et al., (1999)	<ul style="list-style-type: none"> • HS from a black calcareous forest mull • Five wood-degrading basidiomycetes, white-rot type (<i>Bjerkandera adusta</i>, <i>Gymnopilus sapineus</i>, <i>Hypholoma frowardii</i>, <i>Kuehneromyces mutabilis</i>, <i>Pleurotus ostreatus</i>), two ectomycorrhizal fungi (<i>Hebeloma Crustuliniforme</i>, <i>Morchella elata</i>) and three soil-borne microfungi (<i>Botrytis cinérea</i>, <i>Cunninghamella elegans</i>) • 100-mL serum bottles containing 10 mL mineral salt medium or modified Moser medium and HA, inoculated with 10-mm² sectors of precultivated mycelia in air-filtered boxes and incubated at 24°C for 3 weeks. The mineral salt medium consisted of 0.7 g ammonium tartrate, 1 g/L KH₂PO₄, 0.5 g/L MgSO₄·7H₂O, 0.1 g/L NaCl, 0.2 g/L CaCl₂·6H₂O, 4.5 mg/L ZnSO₄·7H₂O, 4 mg/L MnSO₄·H₂O, 4 mg/L FeSO₄·H₂O and 0.5 mg/L thiamine. The modified Moser medium had 5 g/L malt extract, 0.1 g/L yeast extract, 0.5 g/L KH₂PO₄, 0.5 g/L MgSO₄·7H₂O and 0.01 g/L FeSO₄·H₂O. 	<ul style="list-style-type: none"> • In the mineral salt medium, 2 g/L HS were added as the sole source of C. Bleaching took values of 14.8 % for <i>B. Adusta</i>, 12.5% for <i>G. sapineus</i>, 35.9% for <i>H. frowardii</i>, 17.9% for <i>K. mutabilis</i>, 31% for <i>P. ostreatus</i>, 4.9% for <i>H. crustuliniforme</i>, 11.3% for <i>M. elata</i>, 8.1% for <i>B. cinerea</i> and 8.7% for <i>C. elegans</i>. • In the modified Moser medium, 0.8 g/L HS were added. Bleachings of 55.5 % for <i>B. Adusta</i>, 45.9% for <i>G. sapineus</i>, 73.1% for <i>H. frowardii</i>, 41.9% for <i>K. mutabilis</i>, 41.3% for <i>P. ostreatus</i>, 18.7% for <i>H. crustuliniforme</i>, 34.4% for <i>M. elata</i>, 36.7% for <i>B. cinerea</i> and 16.5% for <i>C. elegans</i> were achieved. • Bleaching includes both biosorption and fungal degradation. HS adsorbed to the mycelium were recovered only in the modified Moser medium, resulting in biodegradations 1.3 – 5.4% lower than de decolorizations, except for <i>H. crustiliforme</i> and <i>M. elata</i>, where the fall was higher (a 16% and a 28%, respectively).

Autor	Fungi, medium and experimental conditions	Comments and results
Koukol et al. (2004)	<ul style="list-style-type: none"> • HA from the O_f horizon of a Ferro Humic Podzol in spruce forest, 50.1% C, 3.3% H, 2.6% N, 41.8% O, 2.2% ash and 0.2% P • Saprotrophic ascomycete <i>Chalara longipes</i> strain CCF 3367 • Cotton-plugged 100-mL Erlenmeyer flasks with 50 mL of medium, consisted of 2 g/L NH₄NO₃, 1 g/L K₂HPO₄, 0.5 g/L MgSO₄·7H₂O, 0.5 g/L KCl, 0.01 g/L FeCl₃, 0.5 g/L HA, pH 6.5 and 20 g/L malt extract or 19 g/L glucose, inoculated with 1 mL spores and blended mycelium suspension (2×10³ CFU/mL) and incubated in the dark at 25 °C on a reciprocal shaker (2 Hz) for 21 days. 	<ul style="list-style-type: none"> • Decolorization (as absorbance at 665 nm) and fungal growth for the medium with malt extract were of 75% and 95 mg, respectively. For the medium with glucose, they were of 64% and 38 mg. None is said about the influence of HA adsorption by the cells in bleaching. • The ratio between the absorbances at 465 nm and 665 nm (used as an estimation of the molar-mass changes in HA) was higher in the medium with yeast extract than in the medium with glucose. • Gel permeation chromatography revealed a decrease of the peak corresponding to HA in both media. In the chromatograms of the medium with yeast extract, there also was a decrease of the peaks due to the yeast extract, because the fungus utilized it as feed.

Autor	Fungi, medium and experimental conditions	Comments and results
Řezáčová et al. (2006)	<ul style="list-style-type: none"> • HA and FA from an arable soil (clay-loam orthic luvisol) in Prague. • Soil microfungi <i>Alternaria alternata</i>, <i>Clonostachys rosea</i>, <i>Paecilomyces lilacinus</i> and <i>Phoma</i> sp • 100-mL Erlenmeyer flasks with 10 mL FA or HA solutions, inoculated with 0.5 mL of a homogenized mycelial suspension of each microfungal species and incubated for 1 month. The effect of adding 0.5 mL of glucose solution (200 mg/L) was studied. 	<ul style="list-style-type: none"> • For HA as sole carbon source, the absorbances at 280 nm (related to the amount of phenolics) and at 665 nm (related to decolorization) were decreased by the four fungi, whereas the ratio between the absorbances at 465 nm and at 665 nm (related to changes in the molecular mass of HA) were increased. <i>C. rosea</i> was the most effective in degrading HA, followed by <i>P. lilacinus</i>. None is said about the influence of HA adsorption by the cells in bleaching. The TOC of the solutions was not significant affected by the strains. Biomass growth (as dry mycelial weight) was of 26 – 27 mg for <i>P. lilacinus</i> and <i>Phoma</i> sp. and of 33 – 34 mg for the other two fungi. • For FA as sole carbon source, the four fungal species did not change considerably the absorbance at 665 nm nor the TOC of the solution. Absorbance at 280 nm was significantly reduced only by <i>A. alternata</i> (-9.1%), and the ratio of absorbances at 465 and 665 nm was considerably increased by <i>A. alternata</i> (+107%) and <i>Phoma</i> sp. (+146%) None is said about the influence of FA adsorption by the cells in bleaching. Dry mycelial biomass determined after 1 month was too small to be evaluated. • The effect of glucose was not significant, except in the the ratio of absorbances at 465 and 665 nm for HA.

Autor	Fungi, medium and experimental conditions	Comments and results
Grinhut et al. (2011b)	<ul style="list-style-type: none"> • HA from composted biosolids • White-rot fungus <i>Trametes</i> sp. M23 isolated from composted biosolids and grown on potato-dextrose agar. • 250-mL Erlenmeyer flasks with 50 mL of low nitrogen or high nitrogen medium, 0 or 7 mg/L $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ and 0.4 g/L HA, inoculated with 5 plugs of potato-dextrose agar (5 mm diameter) and incubated in a rotary shaker at 100 rpm and 32°C for 17 days. Both media contained 10 g/L glucose, 0.18 g/L L-phenylalanine, 1 g/L KH_2PO_4, 0.5 g/L KCl, 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 g/L $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.01 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2 mg/L ZnNO_3, 3 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and 0.01 mg/L thiamin, but the high nitrogen medium included 2.1 g/L L-asparagine and the low nitrogen one, 0.3 g/L. 	<ul style="list-style-type: none"> • In the high nitrogen medium with Mn(II), there was no bleaching (reduction of absorbance at 465 nm). MnP activities went through a maximum of 300 U/L at day 7 and then decreased to around 30 U/L at day 17, whereas Lac activity went through a maximum of 2450 U/L at day 14 and took a value of 1850 U/L after 17 days. When Mn(II) was absent, there was no bleaching, MnP activities decreased 7 times and Lac activities slightly increased (and did not went through a maximum). • In the low nitrogen medium with Mn(II), a decolorization of 63% was achieved after 7 days, and then, remained constant. MnP activities went through a maximum of 300 U/L at day 7, fell to 155 U/L at day 10 and remained roughly constant. Lac was almost negligible. In absence of Mn(II), a decolorization of 61% was reached at day 14 and thereafter, and the activities of both MnP and Lac were below 25 U/L. • Glucose was exhausted after 7 days in the high nitrogen medium, and after 14 days in the low nitrogen medium. Its consumption (and therefore, fungal growth) was not affected by the presence or absence of Mn(II). • MnP and Lac activities were considerably different when measured in media without HA (except Lac in the low nitrogen medium, which was similar). LiP was not detected in any case. • None is said about the influence of HA biosorption in the absorbance reduction.

Autor	Fungi, medium and experimental conditions	Comments and results
Zahmatkesh et al. (2016)	<ul style="list-style-type: none"> • Coal HA, containing a 20% small molecules (<0.5 kDa), a 26% medium-sized compounds (0.5 – 1 kDa) and a 54% large substances (1 – 6.5 kDa). • White-rot fungi <i>Pleurotus sajor-caju</i> MES03464, <i>Pleurotus ostreatus</i> MES00036, <i>Trametes versicolor</i> DSMZ 3086 and <i>Phanerochaete chrysosporium</i> DSMZ 1556 • 500-mL flasks containing 150 mL low nitrogen or nitrogen sufficient medium and 400 mg/L HA, inoculated with five pieces of fungal agar, closed with cotton stoppers and incubated at 26°C and 150 rpm for 18 days. Both media contained the same basal solution, minerals and vitamins than Kirk et al. (1978), sodium phthalate buffer (10 mmol/L, pH 4.5), glucose as carbon source (56 mmol/L) and ammonium tartrate as nitrogen source (2 mmol/L in the nitrogen limited medium and 20 mmol/L in the nitrogen sufficient one) 	<ul style="list-style-type: none"> • In the nitrogen sufficient medium, decolorizations (reduction of the chromatographic area) and biomass (weight after drying) were 45.5% and 270 mg for <i>T. versicolor</i>, 74% and 300 mg for <i>P. chrysosporium</i>, 34.2% and 283 mg for <i>P. sajor-caju</i> and 47.9% and 230 mg for <i>P. ostreatus</i>. When the HA adsorbed by mycelium were recovered, decolorizations fell to 32.2% for <i>T. versicolor</i>, 29.2% for <i>P. chrysosporium</i>, 27.8% for <i>P. sajor-caju</i> and 20% to <i>P. ostreatus</i>. • In the nitrogen limited medium, decolorizations and biomass were 70.4% and 212 mg for <i>T. versicolor</i>, 43.4% and 170 mg for <i>P. chrysosporium</i>, 44.3% and 124 mg for <i>P. sajor-caju</i> and 34.9% and 161 mg for <i>P. ostreatus</i>. When the HA adsorbed by mycelium were recovered, decolorizations fell to 47.9% for <i>T. versicolor</i>, 33% for <i>P. chrysosporium</i>, 16.8% for <i>P. sajor-caju</i> and 12.6% to <i>P. ostreatus</i>. • Size exclusion chromatography indicated an increase of the area due to small molecules, and a decrease of the areas of medium and large compounds. Proportions varied with fungi and media. • There was a slight production of FA in almost all the systems, and it was observed that these substances had low affinity to biosorption by fungal hyphae. • Enzyme activities were measured in media with and without HA. The humic compounds increase the Lac activities and decreased the MnP ones. LiP was not detected in any case. <i>T. versicolor</i> showed higher Lac and MnP activity when growing in nitrogen sufficient medium, but showed more HA removal in nitrogen limited medium. <i>P. chrysosporium</i> only generated MnP, being its activities in the nitrogen sufficient medium higher than in the limited one (in accordance with the HA bleaching), but they are lower than those of the <i>T. versicolor</i>, which do not agree with the higher decolorization given by <i>P. chrysosporium</i> in the nitrogen sufficient medium. MnP activities of <i>P. ostreatus</i> (the only enzyme produced by it) were lower than those of <i>P. sajor-caju</i> in both media.

Table S2

Influence of reaction medium and operational conditions in the fungal decolorization of humic compounds.

Autor	Fungi, medium and experimental conditions	Comments and results
Mathur and Paul (1966, 1967)	<ul style="list-style-type: none">The same HA, fungus and experimental conditions than Mathur and Paul (1966, 1967) in Table S1.	<ul style="list-style-type: none">The same than in Table S1: shaking improves the degradation considerably and inoculation with spores led to slightly lower decolorizations than inoculation with mycelium.
Mathur (1969)	<ul style="list-style-type: none">The same FA, fungus and Czapek-Dox broth fortified with 0.02% yeast than Mathur (1969) in Table S1 but with shaking.	<ul style="list-style-type: none">No degradation of FA was observed.
Mathur (1970)	<ul style="list-style-type: none">The same HA, FA, fungus and media than Mathur (1970) in Table S1 but with shaking	<ul style="list-style-type: none">No degradation of FA or HA was detected.

Autor	Fungi, medium and experimental conditions	Comments and results
Dehorter and Blondeau (1992)	<ul style="list-style-type: none"> • HA from forest soil, with 50.2% C, 5.3% H, 3.5% N and 3.8 meq/g COOH (ash-free) • White-rot fungi <i>Phanerochaete chrysosporium</i> BKM-F1767 and <i>Trametes versicolor</i> DSM 3086 • Roux flasks (which promote culture aeration) containing 50 mL of basal medium (Kirk et al., 1978) with 56 mmol/L glucose, 1.2 mmol/L NH₄NO and 0.05% HA, buffered to pH 4.5 with 20 mmol/L sodium tartrate. Sometimes, 1 mmol/L veratryl alcohol was added. <i>P. chrysosporium</i> inocula consisted of filtered conidial suspensions and <i>T. versicolor</i> inocula consisted of mycelial homogenates. Incubation took place for 13 days. 	<ul style="list-style-type: none"> • For <i>P. chrysosporium</i>, the mycelium dry weight increased from 0 mg before the incubation to 52 mg at day 3 and to 115 mg at day 13. The decolorization (determined by absorbance at 350 nm) rose from 20% at day 3 to 61% at day 5, reaching the 73% at day 13. LiP and MnP activities (estimated by absorbance changes instead of by molarity changes) went through maxima of 0.35 U/mL at day 4 and 0.45 U/mL at day 3, respectively. If veratryl alcohol was added, the shape of the activity vs. time curves changed, and two maxima appeared: 0.43 U/mL LiP and 0.6 U/mL MnP at day 4 and 0.88 U/mL LiP and 0.63 U/mL MnP at day 11. • For <i>T. versicolor</i>, the fungal growth was 30 mg at day 3 and 53 mg at day 13, whereas decolorizations of 14% at day 3, 50% at day 5 and 81% at day 13 were observed. LiP and MnP activities went through maxima of 0.40 U/mL at day 4 and 1.10 U/mL at day 3, respectively. If veratryl alcohol was added, all activities are enhanced (the maximum activity of LiP became 1.1 U/mL at 5 days, and, although the maximum of MnP did not vary very much, MnP activities in days 4 – 13 are higher than those without alcohol). • Despite of the increase of LiP and MnP activities due to the alcohol, no significant change in the decolorization was observed. • For <i>T.versicolor</i>, LiP and MnP activities in media without HA were almost zero. For <i>P. chrysosporium</i>, they were lower than those in media with HA. • None is said about the HA adsorbed to the mycelia.

Autor	Fungi, medium and experimental conditions	Comments and results
Ralph et al. (1996)	<ul style="list-style-type: none"> • HA from Morwell brown coal, soluble between pH 3 and 6, with a modal mass around 85 kDa. • The white-rot fungi <i>Merulius tremellosus</i> FPRL 13, <i>Perenniporia tephropora</i> DFP 7904, <i>Phanerochaete chrysosporium</i> ATCC 24725, • 100-mL conical flasks filled with 15-mL or 10-mL low nitrogen medium and 0.045% HA, incubated statically at 28°C for up to 28 days in a humidified atmosphere to minimise evaporation. The medium was a version of that of Tien and Kirk (1988), and contained 2.17 mmol/L N as D-diammonium tartrate, 337 mmol/L C as glucose and tartrate, 2 mmol/L veratryl alcohol, 0.05% w/v oleic acid and 0.005% w/v Tween-80 and was buffered at pH 4.2 with 10 mmol/L 2,2-dimethyl succinate. 	<ul style="list-style-type: none"> • Biomass growth was determined by the weight of dried cells, decolorization by the reduction of absorbance at 400 nm and the variation of the modal relative molecular mass of the remaining HA by size-exclusion chromatography. Biosorbed HA was recovered by alkali washing of the fungi and added to the solutions, but the authors pointed out that not all the HA was recovered. • In 10 mL, <i>M. tremellosus</i> gave 29.8 mg biomass, 43.6% bleaching and 38.9% increase of modal mass; <i>P. tephropora</i> gave 48.2 mg biomass, 28.8% bleaching and 41.1% increase of modal mass; and <i>P. chrysosporium</i> gave 25.4 mg biomass, 49.7% bleaching and a 38.8% decrease of modal mass. In 15 mL, <i>M. tremellosus</i> gave 39.1 mg biomass, 25.1% bleaching and 95.7% increase of modal mass; <i>P. tephropora</i> gave 55.7 mg biomass, 0.0% bleaching and 32.6% increase of modal mass; and <i>P. chrysosporium</i> gave 36 mg biomass, 10.2% bleaching and a 46.7% increase of modal mass. • LiP was detected only for <i>P. chrysosporium</i>, Lac for <i>M. tremellosus</i> and <i>P. tephropora</i> and MnP for the three fungi. Enzymatic activities (measured in media without HA) went through maxima with incubation times. In 10 mL, they were 135 U/L MnP at day 10 and 137.3 U/L Lac at day 28 for <i>M. tremellosus</i>; 45.5 U/L MnP at day 17 and 139 U/L Lac at day 28 for <i>P. tephropora</i>; and 100.9 U/L LiP at day 12 and 476.4 U/L MnP at day 7 for <i>P. chrysosporium</i>. In 15 mL, maxima were 211 U/L MnP at day 18 and 121.5 U/L Lac at day 17 for <i>M. tremellosus</i>; 36 -49 U/L MnP at days 18-28 and 102.4 U/L Lac at day 18 for <i>P. tephropora</i>; and 89 U/L LiP and 633.4 U/L MnP at day 13 for <i>P. chrysosporium</i>.

Autor	Fungi, medium and experimental conditions	Comments and results
Ralph and Catcheside, (1998a)	<ul style="list-style-type: none"> • HA from Morwell brown coal, soluble in water below pH 6 • The white-rot fungus <i>Phanerochaete chrysosporium</i> strain BKM-F 1767 • 100-mL Erlenmeyer flasks with 10 mL low nitrogen medium, 0.03% HA (w/v), buffered at pH 4.2 with 20 mmol/L potassium acetate, inoculated with 10⁵ conidia, plugged with open-cell foam polyurethane stoppers and incubated statically for 20 days at 28 or 37°C under air or hyperbaric oxygen. The medium was the same than Tien and Kirk (Tien and Kirk, 1988), but was supplemented with 0.05% (w/v) oleic acid, 0.005% (w/v) Tween 80 and occasionally 2 mmol/L veratryl alcohol. 	<ul style="list-style-type: none"> • Bleaching was determined by absorbance at 400 nm after recovering the HA adsorbed on the cells by alkali washing. Modal masses were measured by gel permeation chromatography. • At 28°C, hyperbaric oxygen and without veratryl alcohol, decolorization was of 1% at day 3, 97% at day 15 and 95% at day 20. The modal mass of the remaining HA fell a 5% after 9 days when compared with the initial mass and a 48% after 20 days. • At the same conditions, but with veratryl alcohol, decolorization was of 5% at day 3, 47% at day 5 and 96% at day 20. The modal mass of the remaining HA decreased a 3% after 6 days and a 57% after 20 days. • At 28°C, air and veratryl alcohol, decolorization was of 2% at day 3 and of 45% at day 20. The modal mass rose a 15% of the initial value at day 9, but decreased to a 9% of this initial value at day 20. • At 37°C, hyperbaric oxygen and veratryl alcohol, decolorization was of 5% at day 3, 43% at day 5 and 68% at day 20. The modal apparent molecular mass of the remaining HA fell a 6% after 6 days and a 37.5% after 20 days. • At 37°C, air and veratryl alcohol, decolorization was of 11% at day 3 and of 30% at day 20. The modal apparent molecular mass of the remaining HA decreased a 6% after 3 days, increased to a 10% after 6 days and only decreased considerably between days 15 and 20. • LiP and MnP activities were also determined, but in media without HA. They went through maxima at 8 – 12 days, except in the fifth case, where they peaked around 5 – 7 days. MnP activities were higher than those of LiP, especially in the third last cases. Statistical analyses showed that the decline in absorbance correlated well with the time-integrated LiP activity, whereas the modal mass variation was related with the time-integrated MnP activity.

Autor	Fungi, medium and experimental conditions	Comments and results
Ralph and Catcheside (1998b)	<ul style="list-style-type: none"> • HA from Morwell brown coal, soluble in water below pH 6, with a modal mass (determined by gel permeation chromatography) around 71 -73 kDa. • The white-rot fungus <i>Phanerochaete chrysosporium</i> strain BKM-F 1767 • The low-nitrogen medium and the same conditions than Ralph and Catcheside (1994), but with 0, 10, 50 and 200 µmol/L Mn(II) and incubation for 10 days under hyperbaric oxygen. 	<ul style="list-style-type: none"> • Decolorization (decrease of initial absorbance at 400 nm) at day 4 was of 2.8, 10.3, 14.4 and 18.7% for 0, 10, 50 and 200 µmol/L Mn(II), respectively, and at day 10, it was of 63.8, 64.8, 72.0 and 73.6% for 0, 10, 50 and 200 µmol/L, respectively. In all the cases, the HA adsorbed on the mycelia were recovered by alkali washing and added to the solutions. • Modal mass fell with reaction time, but the decrease only was considerable after 5 days. At day 10, masses were of 60 kDa for 0 µmol/L Mn(II), 56 kDa for 10 µmol/L Mn(II) and 46 kDa for 50 and 200 µmol/L Mn(II). • Time-integrated activities of MnP increased with Mn content, whereas those of LiP went through a maximum at 10 µmol/L Mn(II). For concentrations higher than that, time-integrated MnP activities were higher than those of LiP. Measurements in a HA-free media indicated that HA inhibited the activity of both enzymes.
Claus and Filip (1998)	<ul style="list-style-type: none"> • HA from groundwater (Fuhrberg, Germany), with 54.5% C, 4.65% H, 1.9% N and 39.05% O (ash free), 12 µg/mg amino acids and 2.8 µg/mg carbohydrates. • <i>Cladosporium cladosporioides</i> strain P8 • Autoclaved 250-mL Erlenmeyer flasks containing 100 mL of a synthetic culture broth (pH 5.9), with 10 mg/L CuSO₄·5H₂O, • 12.25 g/L KH₂PO₄, 1.42 g/L Na₂HPO₄, 1.7 g/L Bacto yeast nitrogen base (Difco), 10 g/L glucose, 1 g/L NaNO₃ and 1 g/L HA. They were inoculated with 0.1 mL of a spore suspension, and incubated at 25°C for up to 28 days either agitated (100 rpm) or stationary. 	<ul style="list-style-type: none"> • In shaken cultures, the amount of biomass increased to 2800 mg at day 14 and then to 4000 mg at day 28, whereas absorbance at 465 nm decreased a 18% at day 14 and a 40% at day 28. In stationary cultures, biomass rose to 2020 mg at day 14 and then to 3370 mg at day 28, whereas absorbance fell a 15.3% at day 14 and a 28.2% at day 28. Shaking the fungal biomass with NaHCO₃ indicated that around a 30% of the HA was adsorbed by the cells, but it is not clear in the paper if these figure was subtracted to the aforementioned decolorizations. • Activity of the ligninolytic enzymes, estimated by the degradation of an industrial dye, improved with aeration by agitation as well. • At the end of the shaken experiment, the biodegraded HA contained 48.83% C, 4.82% H, 2.88% N and 43.47% O (ash free), 33 µg/mg amino acids and 4 µg/mg carbohydrates. Aromaticity was lower than in the original HA.

Autor	Fungi, medium and experimental conditions	Comments and results
Steffen et al. (2002)	<ul style="list-style-type: none"> • HA extracted from litter from a mixed pine-spruce forest in Finland • The soil-colonizing basidiomycete <i>Collybia dryophila</i> K209 • Stationary culture cultivation was carried out in 1-L Erlenmeyer flasks containing 150 mL of medium (10 g/L of glucose, 2 g/L of KH₂PO₄, 0.5 g/L of MgSO₄·7H₂O, 0.1 g/L of CaCl₂, 0.5 g/L of ammonium tartrate, 2.2 g/L of 2,2-dimethylsuccinate, 0.1 g/L of yeast extract and pH adjusted to 5.0). The concentration of HA was of 250 mg/L. Certain cultures were supplemented with MnCl₂ (0.2 mmol/L). Flasks were inoculated with 10 agar plugs (10 mm in diameter) and incubated at 20°C in the dark for 42 days. 	<ul style="list-style-type: none"> • Without MnCl₂, decolorization (as reduction of absorbance at 450 nm), MnP and Lac activities were of 60%, 43 U/L and 18 U/L, respectively. In the presence of MnCl₂, decolorization, MnP and Lac activities were of 82%, 102 U/L and 23 U/L, respectively. None is said about the biosorption of HA in the cells. Both the decolorization and the enzyme activities rose with incubation time, but the increase rate was higher at the beginning of the reaction than at the end. • Activities of both enzymes determined in a HA-free medium, were lower than those in presence of the humic compound. • Size exclusion chromatography revealed that 60% of the HA was degraded without MnCl₂, whereas 75% was degraded in presence of MnCl₂. Molecular mass distribution of residual HA was changed towards lower masses only in the presence of manganese chloride (from 2.5 to 1.5 kDa for the modal mass). The formation of FA was also studied: FA had a modal mass around 0.5 kDa, and the largest amount of it was found in cultures supplemented with Mn(II)
Steffen et al. (2002)	<ul style="list-style-type: none"> • ¹⁴C-labeled HA by spontaneous oxidative polymerization of ¹⁴C-catechol • The soil-colonizing basidiomycete <i>Collybia dryophila</i> K209 • 15 mL of the same medium than Steffen et al. (Steffen et al., 2002) in Table S1, with 12.8 mg/L HA dissolved in 0.1 NaOH and 0 or 0.2 mmol/L MnCl₂. Incubation was carried out at 24°C in the dark. 	<ul style="list-style-type: none"> • In the absence of Mn(II), the fungus mineralized 18.9% of the HA, while 35.3% was incorporated into the biomass or closely bound to the cell surface, and the release of ¹⁴CO₂ vs. incubation time was almost linear. • Mineralization was faster at the beginning of the reaction than at the end. The presence of Mn(II) caused a noticeable stimulation of ¹⁴CO₂ production (an increase of 49.4%). • In both the presence and absence of Mn(II), only negligible amounts of ¹⁴C-labeled volatile organic compounds were formed.
Grinhut et al. (2011b)	<ul style="list-style-type: none"> • The same HA, fungus, media and experimental conditions than Grinhut et al. (2011b) in Table S1. 	<ul style="list-style-type: none"> • The same than in Table S1: the presence of Mn(II) increases the decolorization rate in the low nitrogen medium, but does not improve very much the final bleaching. The element also increases the activities of MnP in both nitrogen sufficient and nitrogen limited medium, but slightly reduces the activities of Lac.

Autor	Fungi, medium and experimental conditions	Comments and results
Zahmatkesh et al. (2016)	<ul style="list-style-type: none"> • Coal HA • <i>Trametes versicolor</i> DSMZ 3086 • 100-mL flasks containing 25 mL low nitrogen or nitrogen sufficient medium, fungal pellets (8 g wet weight) and an unspecified concentration of HA incubated for 4 days. Occasionally, 1-aminobenzotriazole, an inhibitor of cytochrome P450 enzymes, was added. 	<ul style="list-style-type: none"> • In the nitrogen sufficient medium without inhibitor, decolorizations (absorbances at 450 nm) were 49, 72 and 81% after 1, 2 and 4 days, respectively. With inhibitor, they were of 33% at day 1, of 45% at day 2 and of 80% at day 4. • In the nitrogen limited medium without inhibitor, decolorizations were 52, 78 and 84% after 1, 2 and 4 days, respectively. With inhibitor, they were of 7% at day 1, of 71% at day 2 and of 84% at day 4. • The effect of the adsorbed HA is not referred, but since the authors devoted part of its paper to this issue (see Table S1), it is likely that biosorption was somehow taken into account.

Table S3

Influence of feed concentration in the fungal decolorization of humic compounds.

Autor	Fungi, medium and experimental conditions	Comments and results
Mathur (1969)	<ul style="list-style-type: none"> FA from the Bh horizon of Armadale soil The white-rot fungus <i>Poria subacida</i> 17780 200-mL Erlenmeyer flasks containing 25 mL Czapek-Dox broth fortified with 0.02% yeast extract at pH 5.0, with 0.05 – 1.0% FA, inoculated with 72-h cultures and incubated at 28°C without shaking for 24 or 46 days. 	<ul style="list-style-type: none"> Solutions with FA adsorbed on mycelia served as controls in color comparisons. Decolorizations (reduction of absorbances at 465 nm) after 24 days for 0.05, 0.1, 0.2 and 0.4% FA were 45.3, 26, 0 and 0%, respectively. After 46 days, they only were measured for 0.2 and 0.4% FA, being 32.9 and 13.8%, respectively. For concentrations of 1% FA, there was no decolorization at any time.
Haider and Martin (1988)	<ul style="list-style-type: none"> The same HA, fungus and experimental conditions than Haider and Martin (1988) in Table S1. 	<ul style="list-style-type: none"> The same results than Haider and Martin (1988) in Table S1: for a given concentration of the N sources: the percentage of mineralized HA was not considerably affected by the initial concentration.
Petrovic et al. (1993)	<ul style="list-style-type: none"> HA from a commercial source and HA and FA from the uncultivated soil of a croatian wooded hill Mycelial pellets of the mold <i>Trichoderma viride</i> 100 mL of basal medium (0.2 g/L NH₄NO₃, 1 g/L KH₂PO₄, 0.5 g/L MgSO₄, 0.5 g/L KCl, 0.5 g/L peptone and 0.002 g/L thiamine) with two concentrations of soil HA (40 or 80 mg/L), soil FA (42.5 or 85 mg/L) or commercial HA (25 or 50 mg/L), inoculated with 1 g of mycelia (fresh weight) and incubated at pH 6 and 25°C for 10 days 	<ul style="list-style-type: none"> Commercial HA: For 25 mg/L, bleachings (evaluated by removal of COD) were 30.3% at 5 h, 53.4% at day 3, 60.3% at day 6 and 63.4% at day 10. For 50 mg/L, they were 31.4% at 5 h, 32.8% at day 3, 35.5% at day 6 and 40.3% at day 10. Soil HA: For 40 mg/L, decolorizations were 14.8% at 5 h, 31.6% at day 3, 37.1% at day 6 and 39.9% at day 10. For 80 mg/L, they were 13.1% at 5 h, 35.1% at day 3, 36.1% at day 6 and 37.1% at day 10. Soil FA: For 42.5 mg/L, decolorizations were 11.9% at 5 h, 39.9% at days 3 and 6, and 43.0% at day 10. For 85 mg/L, they were 8.9% at 5 h, 17.1% at day 3, 20.1% at day 6 and 24.6% at day 10. These results comprise both biosorption and fungal decomposition. Further experiments revealed that the removals of the first five hours were due to adsorption, whereas those thereafter were due to biodegradation.

Autor	Fungi, medium and experimental conditions	Comments and results
Willmann and Fakoussa (1997)	<ul style="list-style-type: none"> • HA from german lignite, with 40.3% C, 4% H, 5.1% N, 24.9% O and 25% ash. • Fungal strain RBS 1k, a haploid basidiomycete isolated from an open-cast mining region (Cologne), probably belonging to the <i>Aphyllphorales</i> or <i>Agaricales</i> • 250-mL conical flasks with diluted malt extract and 0.01 – 0.20% w/v HA at 28°C for several days. 	<ul style="list-style-type: none"> • Decolorizations of 58, 65 and 18% were obtained for 0.025, 0.05 and 0.20% HA, respectively, for an unspecified incubation time. These values includes both biodegradation and adsorption. • MnP activities went through maxima with both incubation time and HA concentration. These maxima were of 59 U/L at day 6 for 0% HA, 136 U/L at day 7 for 0.01% HA, 121 U/L at day 7 for 0.025% HA, 173 U/L at day 6 for 0.05% HA, 523 U/L at day 11 for 0.075% HA, 345 U/L at day 11 for 0.1% HA and 205 U/L at day 7 for 0.2% HA.
Fakoussa and Frost (1999)	<ul style="list-style-type: none"> • HA from lignite • <i>Trametes versicolor</i> DSM 3086 • Standing flask cultures with malt extract medium diluted to 1/5 and 0.1 – 4.0 g/L HA in an incubator at 30°C and an atmosphere of 40 vol% oxygen for 7 or 14 days. 	<ul style="list-style-type: none"> • After 7 days, decolorizations of 29% at 0.1 g/L HA, 41% at 0.6 g/L HA, 33% at 1.15 g/L, 24% at 1.55 g/L and 0.5% at 4 g/L HA were achieved. • After 14 days, decolorizations were 57.2% at 0.1 g/L HA, 64.85% at 1.1 g/L HA, 48% at 2.5 g/L and 25.1% at 4 g/L HA. • These values include adsorption and biodegradation.

Autor	Fungi, medium and experimental conditions	Comments and results
Kluczek-Turpeinen et al. (2005)	<ul style="list-style-type: none"> • HA extracted from sieved compost • Compost-dwelling mold <i>Paecilomyces inflatus</i>, strain BKT 02 • 250-mL Erlenmeyer flasks, containing 50 mL culture medium (Czapek-Dox) and 250 or 500 mg/L HA. The basal medium consisted of 2 g/L KH₂PO₄, 0.5 g/L MgSO₄·7H₂O, 0.1 g/L KCl, 0.5 g/L ammonium tartrate, 3 g/L NaNO₃, 0.01 g/L FeSO₄ and 0.05 g/L yeast extract (pH adjusted to 7.0). Inoculation with 0.5 mL of a fungal conidia suspension in water and incubation at 28°C for 31 days 	<ul style="list-style-type: none"> • For 500 mg/L HA, the biomass increased from 0 to 1125 mg in the first 4 days, reached 1350 mg at day 27 and declined to 850 mg at 28 days. For 250 mg/L HA, the numbers were slightly lower. • Lac was the only extracellular oxidoreductase detected. In presence of HA, Lac activity went through a maximum at 11 days and tended to a constant value beyond day 25. The maximum was of 60 U/L for 250 mg/L HA and of 45 U/L for 500 mg/L HA, and the final value of 30 U/L for 250 mg/L HA and of 25 U/L for 500 mg/L HA. In absence of HA, activities were always equal or lower than 5 U/L. • The HA adsorbed on the mycelia was washed by NaOH and added to the solution. Decolorization of HA (measured by absorbance at 450 nm) increased until day 21 and then remained almost constant. For 500 mg/L HA they were of 14, 22 and 33% at days 4, 11 and 21, respectively. For 250 mg/L, they were of 7% at day 4, 10% at day 11 and 30% at day 21. Size exclusion chromatography for 500 mg/L HA showed that the relative concentration of HA (peak area) decreased by about 30%, being the heavier fractions those preferentially attacked. • The production of FA was also studied. The rise of absorbance at 360 nm was of 85% for 500 mg/L HA and of 70% for 250 mg/L HA. Size exclusion chromatography for 500 mg/L HA indicated an increase of only 25% and modest changes in the molecular-mass distribution.

Table S4

Comparison of several fungi or of humic compounds under the same experimental conditions for the bleaching.

Autor	Fungi, medium and experimental conditions	Comments and results
Burges and Latter (1960)	<ul style="list-style-type: none">• HA extracted from the B1 horizon of a podzol soil.• Fungi from the genera <i>Spicaria</i> and <i>Polystictus</i>.• Penicillin flasks with 500 mL of medium, consisting of 0.4 g/L KH_2PO_4, 0.6 g/L K_2HPO_4, 0.5 g/L Mg_2SO_4, 0.5 g/L KCl, 20 g/L sucrose, trace elements, Fries' vitamin solution, 0.2 mg/L vitamin B₁₂, 0.25% agar and 0.039% HA. Flasks were inoculated with either a spore suspension of <i>Spicaria</i> or a mycelial suspension of <i>Polystictus</i>, and incubated at 25°C for six weeks.	<ul style="list-style-type: none">• The loss of HA (by weight) after incubation was 16.92 % for <i>Spicaria</i> and 64.10% for <i>Polystictus</i>. Mycelium were treated with warm 1% potassium phosphate to recover all the adsorbed material.

Autor	Fungi, medium and experimental conditions	Comments and results
Haider and Martin (1988)	<ul style="list-style-type: none"> • HA from a Mollisol (Chernozem near Asel, Germany) bound to ¹⁴C-labelled phenols or anilines (catechol, 4-chlorocatechol, 4,5-dichlorocatechol, aniline hydrochloride, 4-chloroaniline, 2-chlorophenol, 4-chlorophenol and 2,4-dichlorophenol) • <i>Phanerochaete chrysosporium</i> ATCC 32629 • 200-mL Erlenmeyer flasks with 50 mL liquid medium, containing the mineral salts and vitamins of Kirk et al. (1978), 0.56 mmol/L D-glucose as carbon source, 0.6 mmol/L NH₄NO₃ and 0.6 mmol/L L-asparagine as nitrogen sources and 400 mg/L HA, inoculated with spores and held at 35°C for 18 days. 	<ul style="list-style-type: none"> • The chlorinated aromatic moieties were biodegradation intermediates of herbicides and insecticides, which were known to be immobilized in soil by binding to the humus matrix. The main objective of the experiment was to determine if the fungus was able of mineralizing them as well as the non-chlorinated ones, which were known to be units of the HA. • The percentage of HA converted into ¹⁴CO₂ increased with the incubation time. After 18 days it was of 32.5% for catechol, 35.5% for 4-chlorocatechol, 37.8% for 4,5-dichlorocatechol, 20.1% for aniline hydrochloride, 13.4% for 4-chloroaniline, 30.8% for 2-chlorophenol, 17.4% for 4-chlorophenol and 17.1% for 2,4-dichlorophenol. • For the sake of comparison, 0.2 mg of each ¹⁴C-labelled chlorinated compound were added to the 50 mL medium without HA. After 18 days, the free 4-chlorocatechol and the two anilines were more slowly degraded than when linked into the humic matrix, whereas catechol, 2-chlorophenol and 4-chlorophenol were degraded faster. 4,5-dichlorocatechol and 2,4-dichlorophenol displayed almost the same mineralization
Dehorter et al. (1992)	<ul style="list-style-type: none"> • HA from the upper horizon of a forest soil, with two modal masses: 6.8 kDa and 160 kDa. Elementary analysis gave 49.8% C, 5.1% H and 3.2% N (ash free). 26.7% of the C was aliphatic, 26.0% of the C was alkyl C united to –O– (ether or alcohol), 37.5% of the C was aromatic and the remaining was C of carboxylic groups. • <i>P. chrysosporium</i> and <i>T. versicolor</i> in the same conditions than Dehorter and Blondeau (1992), but with 100 mL medium, without veratryl alcohol and incubated at 35°C. 	<ul style="list-style-type: none"> • Reaction was stopped after 30% loss of absorbance at 350 nm (5 – 8 days), but one culture of <i>P. chrysosporium</i> was carried out up to 60%. None is said about the HA adsorbed on the mycelium. • Gel filtration chromatography for HA after 30% bleaching revealed that fractions around 160 kDa were more attacked than those around 6.8 kDa. • ¹³C NMR spectroscopy of the degraded HA indicated that the relative abundance of aromatic C did not vary considerably when compared with that of the original HA, that the results for 60% bleaching with <i>P. chrysosporium</i> were close to those for 30% bleaching, that only <i>P. chrysosporium</i> modified the proportion of carboxylic C (to 8.4 -8.9%), that fungal attack increased the abundance of aliphatic C to 27.1 – 27.6% and that the proportions of alkyl C united to –O– (ether or alcohol) were 26.2 – 26.66% for <i>P. chrysosporium</i> and 24.9% for <i>T.versicolor</i>.

Autor	Fungi, medium and experimental conditions	Comments and results
Ralph et al. (1996)	<ul style="list-style-type: none"> The same HA and experimental conditions than Ralph et al. (1996) in Table S2, but only 15 mL of low nitrogen medium. The same three fungi plus other four white-rot fungi (<i>Ganoderma applanatum</i> DFP 12073, <i>Pycnoporus cinnabarinus</i> FPRL 176, <i>Rigidoporus ulmarius</i> FPRL 241D and <i>Trametes versicolor</i> ATCC 12679) and two brown-rot fungi (<i>Gloeophyllum trabeum</i> DFP 7520 and <i>Lentinus lepideus</i> DFP 2385) 	<ul style="list-style-type: none"> <i>G. trabeum</i> gave 18 mg biomass, 3.1% bleaching and 5.5% decrease in modal mass; <i>L. lepideus</i> gave 47.2 mg biomass, 5.7% bleaching and 2.8% decrease in modal mass; <i>G. applanatum</i> gave 37.7 mg cells, -15.5% decolorization and a 33.7% increase in modal mass; <i>M. tremellosus</i> gave 39.1 mg cells, 25.1% decolorization and 95.7% increase in modal mass; <i>P. tephropora</i> produced 55.7 mg biomass, 0.0% bleaching and 32.6% increase in modal mass; <i>P. chrysosporium</i> gave 36 mg biomass, 10.2% bleaching and 46.7% increase in modal mass; <i>P. cinnabarinus</i> generated 13 mg biomass, -19.6% decolorization and 6.2% increase in modal mass; <i>R. ulmarius</i> generated 38.4 mg cells, 16.4% decolorization and 27.1% increase in modal mass; and <i>T. versicolor</i> generated 42.9 mg cells, -7.4% decolorization and 4.6% increase in modal mass. Enzymatic activities (measured in media without HA) went through maxima with incubation times. LiP was detected only for <i>P. chrysosporium</i>, MnP for <i>M. tremellosus</i>, <i>P. tephropora</i> and <i>P. chrysosporium</i>, and Lac for <i>M. tremellosus</i>, <i>P. tephropora</i>, <i>R. ulmarius</i> and <i>T. versicolor</i>. However, the authors suspected of the presence of enzymes bounded to the cells of <i>G. applanatum</i> and <i>P. cinnabarinus</i> (instead of extracellular ones).
Ralph and Catcheside (1998b)	<ul style="list-style-type: none"> HA from Morwell brown coal, soluble in water below pH 6 The white-rot fungus <i>Phanerochaete chrysosporium</i> strains ME446 (which produces both MnP and LiP) and ME446-B17-1 (which produces only MnP) The low-nitrogen medium and the same conditions than Ralph and Catcheside (1994) in Table S1, with 200 µmol/L Mn(II) and incubation for 16 days under hyperbaric oxygen. 	<ul style="list-style-type: none"> The strains were selected to known if, under hyperbaric oxygen, MnP was responsible of polymerization, and if the combination of both MnP and LiP caused depolymerization. For the ME446, MnP activity was zero at day 3, reached a maximum of 160 U/L at day 6 and decreased to zero at day 14, whereas LiP activity was zero before day 6 and after day 14, peaking at day 8 (86 U/L). For the ME446-B17-1, MnP was zero before day 8, went through a maximum of 90 U/L at day 11 and decreased to 10 U/L at day 16. The modal mass of the residual HA (gel permeation chromatography) and the absorbance at 400 nm fell by 39% and 50% respectively after 16 days

Autor	Fungi, medium and experimental conditions	Comments and results
Fakoussa and Frost (1999)	<ul style="list-style-type: none"> • HA from lignite • The tested fungi were <i>Trametes versicolor</i>, <i>Phanerochaete chrysosporium</i>, • <i>Pycnoporus cinnabarinus</i>, <i>Pleurotus ostreatus</i>, <i>Heterobasidion annosum</i>, <i>Fusarium oxysporum</i>, <i>Paecilomyces farinosus</i>, <i>Lentinula edodes</i> and two unknown strains called RB 18 and RBS 1K. • Cultures were incubated in 100- to 200-mL flasks and shaken with a 20% filling of liquid (malt extract medium diluted to 1/5 and 0.05% HA) at temperatures between 27 and 35°C depending on the strain. Flasks were sealed by silicone sponge stoppers. 	<p>for ME446, whereas for the peroxidase-deficient strain, the mass increased by 48% and the absorbance fell by less than 20%. In the two cases, the HA adsorbed by the mycelia were recovered by alkali washing and added to the solution.</p> <ul style="list-style-type: none"> • Decolorizations (estimated by the decrease of absorbance at 450 nm) were 80% for <i>T. versicolor</i>, 5% for <i>F. oxysporum</i>, 4% for <i>H. annosum</i>, 45% for <i>P. farinosus</i>, 4% for RB 18, 70% for RBS 1K, 2.5% for <i>L. edodes</i>, 48% for <i>P. cinnabarinus</i>, 78% for <i>P. chrysosporium</i> and 52% for <i>P. ostreatus</i>. These values include the effects of both biodegradation and biosorption. • Lac was the dominating enzyme for <i>T. versicolor</i>, <i>H. annosum</i>, <i>P. farinosus</i>, <i>P. cinnabarinus</i> and <i>P. ostreatus</i>, whereas peroxidase was it for <i>F. oxysporum</i>, RB 18, RBS 1K, <i>L. edodes</i> and <i>P. chrysosporium</i>.
Gramss et al. (1999)	<ul style="list-style-type: none"> • HS from a black calcareous forest mull, with a modal mass around 1.3 kDa. • Eight wood-degrading basidiomycetes, white-rot type (<i>Bjerkandera adusta</i>, <i>Clitocybula duseunii</i>, <i>Gymnopilus sapineus</i>, <i>Hypholoma fasciculare</i>, <i>H. frowardii</i>, <i>Kuehneromyces mutabilis</i>, <i>Pleurotus</i> 	<p>Fall of absorbance at 340 nm (%)</p> <p>Biodegradation alone (%)</p> <p>Chromatographic area decrease (%)</p> <p>Modal mass (kDa)</p> <p>Maximum MMO activity (U/L)</p> <p>Maximum Lac activity (U/L)</p> <p>Maximum HRP activity (U/L)</p> <p>Maximum MnP activity (U/L)</p>

Autor	Fungi, medium and experimental conditions	Comments and results									
	<p><i>ostreatus</i>, <i>Stropharia rugoso-annulata</i>), two wood-degrading basidiomycetes, brown-rot type (<i>Fomitopsis pinicola</i>, <i>Piptoporus betulinus</i>), seven terricolous basidiomycetes (<i>Agaricus arvensis</i>, <i>A. bisporus</i>, <i>A. porphyrizon</i>, <i>Clitocybe odora</i>, <i>Coprinus comatus</i>, <i>Lepista nebularis</i>, <i>Marasmius oreades</i>), ten ectomycorrhizal fungi (<i>Amanita muscaria</i>, <i>Hebeloma Crustuliniforme</i>, <i>Lactarius deliciosus</i>, <i>L. deterrimus</i>, <i>L. torminosus</i>, <i>Morchella conica</i>, <i>M. elata</i>, <i>Paxillus involutus</i>, <i>Suillus granulatus</i>, <i>Tricholoma lascivum</i>) and nine soil-borne microfungi (<i>Acremonium murorum</i>, <i>Botrytis cinérea</i>, <i>Chaetomium globosum</i>, <i>Cunninghamella elegans</i>, hyphomycetes G28.2 and G28.30 from lignite, <i>Rhizoctonia solani</i>, <i>Scytalidium lignicola</i>, <i>Trichoderma</i> sp.)</p> <ul style="list-style-type: none"> • 100-mL serum bottles containing 10 mL modified Moser medium (5 g/L malt extract, 0.1 g/L yeast extract, 0.5 g/L KH₂PO₄, 0.5 g/L MgSO₄·7H₂O, 0.01 g/L FeSO₄·H₂O) and 0.8 g/L HS, inoculated with 10-mm² sectors of precultivated mycelia in air-filtered boxes and incubated at 24°C for 3 weeks 	<i>B. adusta</i>	55.5					0.06	0.005	0	9.0
		<i>C. duseñii</i>	56.2	50.1	34.5	0.83	3.7	31.4	0	16.2	
		<i>G. sapienus</i>	45.9	40.6	33.3		0	4.2	0	0	
		<i>H. fasciculare</i>	53.8	51.7	29.2	0.89	0.04	8.9	0.26	49.5	
		<i>H. frowardii</i>	73.1	68.4	51.9	0.85	3.0	6.4	1.9	19.0	
		<i>K. mutabilis</i>	41.9	40.6			1.3	15.3	0.43	7.3	
		<i>P. ostreatus</i>	41.3				8.0	6.3	1.7	8.4	
		<i>S. rugoso-annulata</i>	56.7	44.8	47.3	0.87	1.08	7.2	1.2	34.2	
		<i>F. pinicola</i>	72.3	47.1	54.3	1.02	0	0	0	0	
		<i>P. betulinus</i>	71.5	49.5	52.2	1.05	0	0	0	0	
		<i>A. arvensis</i>	25.1				4.9	1.5	0	0	
		<i>A. bisporus</i>	26.3				19.0	16.2	0	0.58	
		<i>A. porphyrizon</i>	32.9	24.4	5.5	1.3	7.6	11.9	2.0	0.07	
		<i>C. odora</i>	28.7				2.0	7.5	0.36	0	
		<i>C. comatus</i>	32.3				5.4	3.9	0.53	0.05	
		<i>L. nebularis</i>	31.6	26.9	15.5	1.3	1.3	4.4	0.49	0	
		<i>M. oreades</i>	15.7				0.46	0.12	0	0	
		<i>A. muscaria</i>	12.7	6.3			0.1	0.06	0	0	
		<i>H. crustuliniforme</i>	18.7	2.6	38.7	1.3	0	0	0	0.15	
		<i>L. deliciosus</i>	17.5	8.5	7.7	1.3	0.47	0.58	0.56	0	
		<i>L. deterrimus</i>	13.9				1.5	30.3	2.6	0	
		<i>L. torminosus</i>	8.1				20.0	22.2	2.06	0	
		<i>M. conica</i>	31.8	20.1			2.7	2.0	0.39	0.48	
		<i>M. elata</i>	34.4	6.1	27.5	1.3	0.31	0.57	0.09	0.05	
		<i>P. involutus</i>	11.7	18.2	38.6	1.3	0	0.5	0.83	0	
		<i>S. granulatus</i>	2.9	7.5	0	1.3	0	0.023	0	0	
		<i>T. lascivum</i>	18.3				0.08	0.35	0.34	0	
		<i>A. murorum</i>	25.6				0	0	0	0	
		<i>B. cinerea</i>	36.7	31.3	30.8	1.3	0.18	0.015	0.03	0	
		<i>C. globosum</i>	2.5				0	0	0	0	
	<i>C. elegans</i>	16.5	14.5	27.9	1.3	0	0	0	0		
	G 28.2	1.8				0	0.004	0.47	0		
	G. 28.30	25.9				0	0	0	0		
	<i>R. solani</i>	21.1				0.2	0	0	0		
	<i>S. lignicola</i>	77	70.9	61.8	0.97	0.58	0.12	0.13	0		
	<i>Trichoderma</i> sp.	25.4				0	0	0	0		

Temp et al. (1999)

- HS from german lignite. The humic fraction had a modal mass of 2.76 kDa, and the fulvic fraction a modal mass around 1 – 2 kDa.
- Decolorizations (as reductions of the absorbance at 465 nm) were of 25% at 8 days, 75% at 18 days and of 94% beyond 18 days for JF 596, of 26% at 8 days, 49% at 10 days and 71% at 20 days for *P. cinnabarinus* and of 7% at

Autor	Fungi, medium and experimental conditions	Comments and results
	<ul style="list-style-type: none"> • The white-rot fungi <i>Pycnoporus cinnabarinus</i> (ATCC 200478), <i>Polyporus ciliatus</i> and an unidentified white-rot basidiomycete JF 596, isolated from decaying soft wood in Thuringa • Mycelia from 10 agar blocks (0.5 cm x 0.5 cm) taken from the edge of actively growing colonies cultured on malt extract agar plates were used as inoculum for 250 mL Erlenmeyer flasks containing 75 mL of liquid modified Dodson medium and 1.7 g/L HS. The pH of the medium was adjusted to 4.6 with 1 M NaOH. The strains were grown in shaken cultures (140 rpm) for • 20 days at 24°C. 	<p>5 days and of 41% beyond 18 days for <i>P. ciliatus</i>. Adsorption to mycelial pellets was analyzed, but it resulted to be negligible.</p> <ul style="list-style-type: none"> • JF 596 produces Lac, MnP and LiP. <i>P. Cinnabarinus</i> only generates Lac. <i>P. ciliatus</i> produces both Lac and MnP. See Figure 2. • Gel permeation chromatography of HA after 20 days revealed a decrease of the chromatographic area of 72% for JF 596, of 58% for <i>P. cinnabarinus</i> and of 34% for <i>P. ciliatus</i>. The modal mass in the HA slightly decreased for JF 596 but increased a little for <i>P. cinnabarinus</i> and <i>P. ciliatus</i>. • The production of FA was also investigated. The increase in the chromatographic area of FA was of 161% for JF 596, 101% for <i>P. cinnabarinus</i> and of 14% for <i>P. ciliatus</i>. The modal mass of the FA remained unchanged for the three fungi.
Yanagi et al. (2002)	<ul style="list-style-type: none"> • HA from five Japanese soils (Umbri Andosol 1 and 2, Dystric Cambisol 1 and 2 and Fibric Histosol) • Three fungi (<i>Corioulus consors</i> IFO 9078, <i>Coriolus hirsutus</i> IFO 4917 and <i>Lenzites betulina</i> IFO 6266) isolated from the soil belonging to Umbric Andosol.) • Test tubes containing 10 mL of Czapek-Dox medium with 0.05% w/v HA, pH adjusted to 6.8, inoculated and kept at 25°C for 21 days in stationary incubators. 	<ul style="list-style-type: none"> • Cambisol HA were characterized as having higher content of carbohydrate C, lower contents of aromatic C and lower degree of aromaticity than Andosol HA and Histosol HA. • Decolorizations were evaluated by absorbance at 600 nm. None is said about the HA adsorbed to the cells. <i>C. consors</i> gave 25.9% for Andisol 1, 27.7% for Andisol 2, 29.3% for Histosol, 41.6% for Cambisol 1 and 51.1% for Cambisol 2. <i>C. hirsutus</i> gave 2.6% for Andisol 1, 31.1% for Andisol 2, 9.9% for Histosol, 37.3% for Cambisol 1 and 45.5% for Cambisol 2. <i>L. betulina</i> gave -0.9% for Andisol 1, 17.5% for Andisol 2, 2.6% for Histosol, 11.8% for Cambisol 1 and 50.6% for Cambisol 2.
Yanagi et al.	<ul style="list-style-type: none"> • Soil HA from 15 Japanese sites (7 Andisols, 4 Inceptisols, 2 Ultisols, 1 Mollisol and 1 Histosol) 	<ul style="list-style-type: none"> • The HA adsorbed by the cells was recovered by NaOH washing, and added to the solutions. Decolorizations (evaluated by absorbance reduction at 600

Autor	Fungi, medium and experimental conditions	Comments and results
(2003)	<ul style="list-style-type: none"> • White-rot fungus <i>Coriolus consors</i> IFO 9078 • Test tubes containing 5 mL Czapek-Dox liquid medium at pH 6.8 and 0.25 g/L HA, inoculated with a loopful of fungal hyphae from a slant culture on popato sucrose agar and kept at 25°C for 14 days in stationary incubators 	<p>nm) were 18.8% for the Andisol HA from Gyoseizan, 22.2% for the Andisol HA from Hachibuse, 19.3% for the Andisol HA from Inogashira, 19.9% for the Andisol HA from Makino, 8.8% for the Andisol HA from Oginosen, 18.1% for one Andisol HA from Sugadaira (grassland), 31.8% for other Andisol HA from Sugadaira (forest), 39.5% for the Inceptisol HA from Dando, 36.2% for the Inceptisol HA from Hanaore, 38.2% for the Inceptisol HA from Keirozan, 29.5% for the Inceptisol HA from Nagamine, 23.9% for the Ultisol HA from Andover, 23.2% for the Ultisol HA from Miyagi, 18.0% for the Mollisol HA from Wakau and 18.4% for the Histosol HA from Ichijima.</p> <ul style="list-style-type: none"> • Absorbances, aromaticities (determined by ¹³C-nuclear magnetic resonance), total acidities, elemental compositions (C,H, N, O) and atomic ratios (H/C, O/C, N/C, O/H from the elemental compositions) for the HAs before the reaction were measured, in order to correlate the decolorization with some of these properties.
Kabe et al. (2005)	<ul style="list-style-type: none"> • HA from german lignite • Three white-rot fungal strains (FTN, WO-4, and WT-5) isolated from decaying woods of Japan, but not identified taxonomically. • 100-mL Erlenmeyer flasks containing 10 mL of the basal medium of Willmann and Fakoussa (1997) at pH 5.6, supplemented with 50 ppm veratryl alcohol and 1000 ppm HA, inoculated with 0.5 mL of cell suspensions and incubated statically at 28°C for 20 or 63 days. 	<ul style="list-style-type: none"> • Adsorbed HA was recovered by NaOH washing and added to the solutions. Absorbances at 480 and 275 nm served to calculate decolorizations and depolymerizations, respectively. • For FTN, decolorizations were of 50% at 7 days and of 73% at 20 days, whereas depolymerizations were 22% at day 7, 32% at day 15 and 26% at day 20. • For WO-4, decolorizations were of 56% at 7 days, 67% at 27 days, 71% at 56 days and 84% at 63 days, whereas depolymerizations were 29% at day 14, 38% at day 27, 39% at day 56 and 55% at 63 days. • For WT-5, decolorizations were of 47% at 7 days and of 70% at 56 days, whereas depolymerizations were 24% at day 7, 32% at day 15 and 38% at day 56. From day 56 to day 63, both parameters slightly decreased. • Activities of Lac, LiP, MnP and AAO were determined in a HA-free medium. For FTN, MnP alone correlated well with the adsorbance reductions. For the other two fungi, the combination of both MnP and Lac

Autor	Fungi, medium and experimental conditions	Comments and results
Belcarz et al. (2005)	<ul style="list-style-type: none"> • HA from chernozem (51.4% C, 3.96% H, 3.58% N, 2.87% ash, 3.296% phenolics) and lessive soil (51.54% C, 4.41% H, 2,9% N, 4.14% ash, 3.132% phenolics) • <i>Bjerkandera adusta</i> R59, a white-rot fungus isolated from black earth soil. • Erlenmeyer flasks (300 mL) containing 100 mL of Fahreus medium with 0.07% glucose and 0.03% HA. Sterile conditions. 2 mL of inoculum. Reaction for 21 days at 26°C 	<p style="text-align: center;">could explained the results.</p> <ul style="list-style-type: none"> • Decolorization (measured by changes in absorbance at 450 nm) was moderate in HA from lessive soil (0% at 1 day, 42% at 7 days and 58% at 21 days) and low in HA from chernozem (0% at 1 day, 22% at 7 days and 37% at 21 days). None is said about the HA adsorbed to the mycelium. • Extracellular peroxidase activities were slightly higher for HA from lessive soil than for HA from chernozem, and went through a maximum around 10 mU/L at 14 days. Lac activities were negligible in both cases. • Concentrations of phenolic compounds (as vanillic acid, measured at 500 nm) were 8.24 mg/L for media with HA from chernozem and 7.83 mg/L for HA from lessive soil. They quickly decreased in the first day of reaction, being below 2 mg/L between days 1 and 21.
Řezáčová et al. (2006)	<ul style="list-style-type: none"> • FA from an arable soil (clay-loam orthic luvisol) in Prague. • Eight soil microfungi (<i>Alternaria alternata</i>, <i>Clonostachys rosea</i>, <i>Paecilomyces lilacinus</i>, <i>Phoma</i> sp, <i>Penicillium canescens</i>, <i>Exophiala</i> cf. <i>salmonis</i>, <i>Fusarium caeruleum</i> and <i>Fusarium redolens</i>) and two white-rot fungi(<i>Phanerochaete chrysosporium</i> and <i>Trametes versicolor</i>) • 100-mL Erlenmeyer flasks with 10 mL FA solution and 0.5 mL mineral solution, inoculated with 0.5 mL of a homogenized mycelial suspension of each microfungal species and incubated for 1 month. Mineral stock solution contained 2920 mg/L $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 1957 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 972 mg/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 704 mg/L Na_2SO_4, 193 mg/L KNO_3, 45 mg/L K_2HPO_4, 25 mg/L NH_4NO_3 and 6 mg/L KH_2PO_4 	<ul style="list-style-type: none"> • Decolorization (as reduction of absorbance at 665 nm) or change of the molecular mass of the FA (evaluated by the ratio of the absorbances at 465 nm and 665 nm) were not significant for any fungus. Reduction of the amount of phenolics (as decrease of absorbance at 280 nm) was only important for <i>P. lilacinus</i> (-10.4%) and <i>T. versicolor</i> (-11.8%). None is said about the influence of FA adsorption by the cells in the absorbance reduction. The amount of biomass produced was of around 75.3 mg for <i>F. caeruleum</i>, 55.5 mg for <i>Phoma</i> sp. and <i>E. cf. salmonis</i>, 45 mg for <i>P. canescens</i> and <i>F. redolens</i>, 34- 37 mg for <i>A. alternata</i>, <i>C. rosea</i> and <i>P. lilacinus</i>, 8.8 mg for <i>P. chrysosporium</i> and 2.4 mg for <i>T. versicolor</i>, • Average values of MnP activities from the third to the 28th days of incubation were 0.66 U/L for <i>A. alternata</i>, 0.45 U/L for <i>C. rosea</i>, 0.43 U/L for <i>P. lilacinus</i> and 0.91 U/L for <i>Phoma</i> sp. Average values for Lac and other peroxidases were of 0.02 – 0.04 U/L, except for <i>C. rosea</i> (0.11 U/L Lac), <i>P. lilacinus</i> (0.07 U/L Lac) and <i>Phoma</i> sp. (0.15 U/L other peroxidases). The TOC of the remaining solutions with these four soil fungi did not considerably differ from that of the initial FA solutions.

Autor	Fungi, medium and experimental conditions	Comments and results
Kornilowicz-Kowalska et al. (2008)	<ul style="list-style-type: none"> • HA from brown coal, chernozem and lessive soil • Anamorphic basidiomycete <i>Bjerkandera adusta</i> R 59 strain, isolated from a black earth soil. • 100-mL Erlenmeyer flasks were filled with 50 mL Park and Robinson medium containing 0.01% HA and 0.25% glucose, inoculated with 1 mL of homogenized mycelium (10⁵ CFU/mL) and stationarily incubated at 26°C up to 60 days 	<ul style="list-style-type: none"> • The decolorization of HA from chernozem, brown coal and lessive soil on day 7 corresponded to 60, 38 and 17% loss of their initial absorbance at 400 nm, respectively. Thereafter, bleaching augmented steadily and after 60 days it finally reached 90.5, 83 and 74% for HA from chernozem, brown coal and lessive soil, respectively. The initial strong decrease in the HA from chernozem was not due to adsorption, which did not exceed 6% of total HA removal. • Growth of <i>B. adusta</i> was assessed qualitatively by observing the formation of aerial mycelium. • Peroxidase activities went through maxima with time. These maxima were of 13.27 U/L at 21 day for brown coal, 11.95 U/L at day 35 for lessive soil and 7.7 U/L at day 28 for chernozem. At the end of the experiment, the enzyme activities dropped to values less than 10% of its maximum value • Methoxyphenolic or vanillic acid levels (as absorbance at 500 nm) for HA from lessive soil were of 100, 76 and 70% at days 10, 24 and 60, respectively; for HA from chernozem were of 90, 89, 69 and 63% at days 3, 14, 24 and 60, respectively; for HA from brown coal were of 85, 84, 76 and 70% after 3, 24, 28 and 60 days.
Grinhut et al. (2011a)	<ul style="list-style-type: none"> • HA from composted biosolids, with 48.8% C, 5.6% H, 7.5% N, 36%O and 2.1% S (ash-free) and a modal mass around 10.7 kDa. • White-rot fungi <i>Trametes</i> sp. M23 and <i>Phanerochaete</i> sp. Y6 isolated from composted biosolids and grown on potato-dextrose agar. • The same low nitrogen medium and experimental conditions than Grinhut et al. (2011b), incubated for 8 days. 	<ul style="list-style-type: none"> • Decolorizations (estimated by absorbance at 465 nm) of 52% at day 5 and 63% at day 8 for <i>Trametes</i> sp. M23 and of 41% at day 3 and 78% at day 8 for <i>Phanerochaete</i> sp. Y6 were achieved. None is said about adsorption. • Size exclusion chromatography after 8 days revealed a preferential attack to the high-size fraction. The modal mass for the <i>Trametes</i> sp. M23 was around 4.5 kDa. The distribution achieved by <i>Phanerochaete</i> sp. Y6 was roughly bimodal (peaks at 3 and 0.4 kDa). • Elemental analysis after 8 days indicated an increase in the %O and a decrease of %C and %N. The O increase was higher for <i>Trametes</i> sp. M23 than for <i>Phanerochaete</i> sp. Y6. • Proton nuclear magnetic resonance displayed a decrease in aromaticity and

Autor	Fungi, medium and experimental conditions	Comments and results
		<p>an increase of oxygen-containing groups (specially, for <i>Trametes</i> sp. M23)</p> <ul style="list-style-type: none"> • Fourier transform cyclotron resonance mass spectrometry showed consecutive series of masses linked to biochemical pathways such as demethylation, side chain oxidation, hydrolysis and aromatic ring cleavage.

Table S5.

Operational features of some fungal decolorizations, not previously cited.

Autor	Fungi, medium and experimental conditions	Comments and results
Mathur and Paul (1966, 1967), Paul and Mathur (1967)	<ul style="list-style-type: none"> • HA from the Ap horizon of Melfort Orthic Black soil, with 54% C, 4.76% H, 3.89% N and 37.4% O (ash-free) • <i>Penicillium frequentans</i> from the same soil • 0.02% HA in a Czapek Dox broth without sucrose. Inoculation with mycelium and incubation for 7 weeks at room temperature with shaking. 	<ul style="list-style-type: none"> • In the shaken culture inoculated with mycelium, the absorbance at 465 nm decreased a 31.6% when HA was the source of C. This bleaching was only caused by biodegradation. • The partially degraded HA contained 54.2% C, 4.90% H, 4.74% N and 36.2% O (ash-free). Infrared spectra showed a considerable increase in the hydroxyl content and a moderate increase in the phenolic hydroxyl and the carboxyl groups. • The ratio between the absorbances at 465 and 665 nm, increased a 11.6%, which indicated a decrease of the aromaticity (and therefore, a preferential attack to the aromatic portion of the HA) • Gel chromatography indicated that molecular weights of HA varied from less than 5000 to more than 50000 and that the fungus preferred to degrade moieties larger than 50000. However, further experiments separated the HA in four fractions (>35000, 8000 – 35000, 6000 – 8000 and 6000<), and found that the lighter fraction was the most susceptible of attack (followed by the heaviest fraction). This suggested that, in the mixture, the larger molecules were not fully decomposed, but converted to intermediate and small-sized molecules.

Autor	Fungi, medium and experimental conditions	Comments and results
Willmann and Fakoussa (1997)	<ul style="list-style-type: none"> The same HA, fungal strain and malt extract medium described in Table S3, but with 0.05% HA 	<ul style="list-style-type: none"> Decolorization of 82% was reached after 13 days, but most of it happened between days 6 and 7 (increase from 20% to 65%). These values includes both adsorption and biodegradation. MnP activities were of 20, 35, 82, 116 and 39 U/L at days 5, 6, 10, 11 and 13, respectively. Gel permeation chromatography indicated a decrease of the modal mass. ¹³C-nuclear magnetic resonance revealed an increase of carboxylic groups, hydroxylated and methoxylated aliphatic groups and a decrease in aromatics. Elemental analysis showed a high increase of N and O, a moderate rise of C and H and a decrease of ash content.
Fakoussa and Frost (1999)	<ul style="list-style-type: none"> HA from lignite, with a 20% high molecular weight fractions and a 75% low molecular weight fractions (determined by gel permeation chromatography) <i>Trametes versicolor</i> DSM 3086 Standing flask cultures with malt extract medium diluted to 1/5 and 0.85 g/L HA in an incubator at 30°C and an atmosphere of 40 vol% oxygen for 16 days. 	<ul style="list-style-type: none"> For HA, absorbances at 280 nm decreased a 5.8% after 3 days, a 63% after 6 days and a 75% after 16 days, whereas absorbances at 450 nm decreased a 7.8% after 3 days, a 72% ater 6 days and a 86% after 16 days. In principle, these bleachings included the effect of both adsorption and fungal degradation. FA production was evaluated as well. Absorbances at 280 nm increased a 0% at day 3, a 245% at day 6 and a 322% at day 16, whereas absorbances at 450 nm increased a 0% at day 3, a 515% at day 7 but a 415% after 16 days. Gel chromatography revealed that most of the conversion took place between days 2 and 6. At the end of the experiment, low molecular weight fractions were around 98%, and their modal mass had decreased when compared to that of the untreated HA. Lac activities were of 15 and 25 U/L at days 0 and 16, respectively, going through a maximum of 425 U/L at day 6. Biomass dry weights of 75 and 85 mg were obtained at days 3 and 7, respectively, but after day 11, the weights oscillated between 58 and 70 mg.

Autor	Fungi, medium and experimental conditions	Comments and results
Wunderwald et al. (2000)	<ul style="list-style-type: none"> • Fluorinated HA, synthesized by spontaneous oxidative polymerization of 3-fluorocatechol • White-rot fungus <i>Nematoloma frowardii</i> b19 • Flasks containing 20 mL liquid (0.25 g/L HA, 5 g/L glucose and 0.025 g/L MnCl₂), inoculated with three agar plugs (diameter 9 mm) of active mycelium and incubated at 24 °C on a rotary shaker (140 rpm) in the dark for 24 days. 	<ul style="list-style-type: none"> • Decolorizations (as reduction of absorbance at 450 nm) and MnP activities were around 10% and 93 U/L at day 4, 42% and 155 U/L at day 8, 48% and 350 U/L at day 10, 69% and 407 U/L at day 15 and 77% and 745 U/L at day 24. Lac activity was negligible throughout the experiment and did not exceed 36 U/L. • It is stated that there was no absorption of HA to the fungal hyphae because they displayed a light color throughout the experiment.

Table S6

Decolorization of humic compounds by means of fungal enzymes alone.

Autor	Fungi, medium and experimental conditions	Comments and results
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Autor	Fungi, medium and experimental conditions	Comments and results
Wondrack et al. (1989)	<ul style="list-style-type: none"> • HA from North Dakota lignite and German subbituminous coal, both coals previously treated with nitric acid for improving the biodegradation • LiP from the white-rot fungus <i>Phanerochaete chrysosporium</i> Burds ME-446 • Capped tubes with 1 mL mixture (buffer, 0.1 mmol/L MnSO₄, 0.1% Tween 20, 200 – 1000 U/L LiP, 0.45 – 1.0 mmol/L H₂O₂ and 30 or 300 mg/L HA solution), flushed with 100% oxygen and incubated at 37°C. The buffer was sodium tartrate (20 mmol/L, pH 3) or dimethylsuccinate (10 mmol/L, pH 4.5). In one experiment, veratryl alcohol was added. 	<ul style="list-style-type: none"> • Gel permeation chromatography was the only technique used. • 300 mg/L HA from North Dakota lignite, incubated with sodium tartrate buffer, 900 U/L LiP and an unspecified concentration of H₂O₂ for 4 h generated a peak of lower area than that representing the non-incubated polymer, broader and with a lower modal mass. However, there was no formation of low molecular weight fragments. Incubation with dimethylsuccinate buffer, 4500 U/L LiP and 0.6 mmol/L H₂O₂ (added in 0.15 mmol/L portions at 0, 2, 4 and 6 h) led to much less total degradation after 4 and 24 h, but generated many new components of both higher and lower molecular weight than the starting material. • 30 mg/L HA from German subbituminous coal, incubated with 10000 U/L LiP, 0.45 mmol/L H₂O₂, oxygen every 15 min and an unspecified pH for 1 h, also generated a peak with lower area than that of the non-incubated HA, but there were not new low molecular weight peaks. When the HA concentration was increased to 300 mg/L, a major disappearance of coal polymer takes place in 8 h. If 2 mmol/L veratryl alcohol was added, there was a near total disappearance of the coal polymer peak after 8 h.
Willmann and Fakoussa (1997)	<ul style="list-style-type: none"> • HA from german lignite • Purified extracellular MnP of the fungal strain RBS 1k • Flasks containing 500 U/L MnP, 0.05% HA, 3 mmol/L H₂O₂ and 0.15 mmol/L MnSO₄ under unspecified conditions. 	<ul style="list-style-type: none"> • In the same paper, it was found that the fungus RBS 1 k was able of degrading the HA. • However, no bleaching or molecular mass reduction of the HA took place when the enzyme alone was used. Then, the authors supposed that the degradative activity of the basidiomycete RBS 1k was bound to the cell wall or acted only near the cell wall, and that the adsorption of HA molecules onto the fungal hyphae might be required for their biodegradation

Autor	Fungi, medium and experimental conditions	Comments and results
Hofrichter and Fritsche (1997a)	<ul style="list-style-type: none"> • HS from german lignite. The humic fraction had a modal mass around 2.5 kDa and the fulvic fraction, around 0.7 kDa. • MnP from the white-rot fungus <i>Nematoloma frowardii</i> b19. • 25-mL culture flasks containing 10 mL reaction mixture on a rotary shaker (160 rpm) at 28 °C in the dark over a period of 168 h. The reaction solution consisted of 61 mmol/L sodium malonate buffer (pH 4.5), 250 mg/L HA, 2.5 mmol/L MnCl₂, 30.1 mmol/L glucose, 20 U/L glucose oxidase (from <i>Aspergillus niger</i>, low in catalase) continuously generating H₂O₂ at the rate of 20 nmol/min/ml during the course of reaction, 0.6 mmol/L glutathione, 783 mmol/L dimethylformamide and enzyme crude extract corresponding to 800 U/L MnP 	<ul style="list-style-type: none"> • Degradation of the lignite HS by the <i>Nematoloma frowardii</i> b19 was seen in an agarized medium by the authors (Hofrichter and Fritsche, 1997b). The fungus produced Lac, LiP and MnP, but only the last one was selected, because it high stability. • Enzyme activity decreased to 590 U/L immediately after addition to the liquid culture (due to inhibition because the HS) and remained almost constant during the 168 h. • For the humic fraction, the absorbance at 360 nm decreased a 28% after 10 h and a 62% after 168 h, whereas absorbance at 450 nm decreased a 36% at 10 h and a 73% at 168 h. • For the fulvic fraction, absorbance at 360 nm increased a 61% after 10 h and a 95% after 168 h, but at 450 nm, it increased a 130% after 10 h, and slightly decreased from 24 to 168 h. • Gel permeation chromatography confirmed the changes in absorbance: degradation of HA and formation of FA were most effective within the first 10 h of the reaction. It also was seen that repolymerization reactions took place in HA, because the apparition of a peak around 30 kDa at 10 h. However, the peak disappeared as the reaction progressed.

Autor	Fungi, medium and experimental conditions	Comments and results
Hofrichter et al. (1998)	<ul style="list-style-type: none"> • Synthetic ^{14}C-labelled HA prepared from ^{14}C-catechol by spontaneous polymerization or by Lac-catalyzed polymerization. The modal mass of the spontaneously polymerized one was around 3.5 kDa. • MnP from the white-rot fungus <i>Nematoloma frowardii</i> b19. • Sterile 10-mL tubes tightly closed with rubber septa and sealed with plastic screw caps. The tubes contained the following sterilized components in a total of 1 ml: 30 mmol/L sodium malonate buffer (pH 4.5), 1 mmol/L MnCl_2, 15 mmol/L glucose, 40 U/L glucose oxidase (from <i>Aspergillus niger</i>, low in catalase) to generate H_2O_2, 0, 1 or 10 mmol/L glutathione, and 2000 U/L MnP. HA was added in 0.1 M NaOH to a final radioactivity of 1682 Bq. The samples were incubated at 37 °C on a rotary shaker (160 rpm) for 144 h. 	<ul style="list-style-type: none"> • The percentage of HA converted to $^{14}\text{CO}_2$ increased with time, but the highest amounts of carbon dioxide were released within the first 24 h of incubation. After 144 h, it was of around 16% for 0 mmol/L glutathione, 43% for 1 mmol/L glutathione and 50% for 20 mmol/L glutathione. The polymerization of the HA (spontaneous or catalyzed by Lac) did not considerably influence the results. • A sample of the HA generated by spontaneous polymerization and treated with 10 mmol/L glutathione for 144 h was subjected to gel permeation chromatography, and it was observed that there was decrease of the modal mass from 3.5 kDa to 1.5 kDa and the formation of low-molecular-mass products (apparition of a peak below 0.25 kDa). Furthermore, the total radioactivity in the reaction mixture decreased by about 50%
Ziegenhagen and Hofrichter (1998)	<ul style="list-style-type: none"> • HA from german lignite • MnP from the white-rot fungus <i>Clitocybula dussenii</i> b11 • 10-mL flasks containing 4 mL of the reaction mixture on a rotary shaker (180 rpm) for 24 h. The mixture contained sodium malonate (50 mmol/L, pH 4.5) as basal buffer, 5 – 500 mg/L HA, 0 -10 mmol/L glutathione, 0 – 5% v/v <i>N,N</i>-dimethylformamide and 0 – 10% dimethylsulfoxide. 	<ul style="list-style-type: none"> • The medium also contained 1 mmol/L MnCl_2 and H_2O_2 continuously generated by glucose oxidase of <i>Aspergillus niger</i> at a rate of 0.046 mmol/min. The incubation temperature was of 37°C. These three conditions were optimized previously for MnP in media without HA. • The highest decolorizations (reduction of absorbance at 450 nm) were of 50% for 250 mg/L HA, of 67% for 250 mg/L HA + 0.6 mmol/L glutathione and of 71% for 250 mg/L HA + 0.6 mmol/L glutathione + 4% <i>N,N</i>-dimethylformamide. Dimethylformamide performed better than dimethylsulfoxide. • Gel permeation chromatography indicated a decrease of the amount of HA and of its modal mass as the incubation time increased (see Figure 1a). The production of FA was also monitored, and both its concentration and modal mass became higher.

Autor	Fungi, medium and experimental conditions	Comments and results
Ralph and Catcheside (1998b)	<ul style="list-style-type: none"> • HA from Morwell brown coal, soluble between pH 3 and 4.5 • Commercial MnP from the white-rot fungus <i>Phanerochaete chrysosporium</i> • Dialysis tubing (membranes of 12 kDa) containing 0.14 mg HA in 1 mL lactate buffer (0.5 mol/L sodium lactate, pH 4.5) was suspended in an external solution of 5 mL lactate buffer with 5 units partially purified MnP, 1 mmol/L H₂O₂ (added at a rate of 0.05 mL/h for 6 h with constant stirring) and 1 mmol/L MnSO₄, and incubated at 30 °C for 24 h. 	<ul style="list-style-type: none"> • Neither the modal apparent relative mass nor the absorbance changed when MnP, H₂O₂ or Mn (II) was omitted from the external solution. • When all the reactants were present, the modal mass of the HA increased by 18%, but no change was observed in the absorbance at 400 nm. MnP activity was not detected within dialysis tubing, indicating that the membrane remained impermeable to the enzyme and that the transformation was due to a small species generated in the external solution. • Further experiments with Mn(III) and HA in a malonate buffer under air, oxygen and a mixture of 5% H₂ + 95% N₂ pointed out that this species, produced by the oxidation of Mn(II) by the MnP, was the responsible of the polymerization of HA under certain atmospheres (air and H₂+N₂), but that it also could depolymerize HA under other ones (oxygen)

Autor	Fungi, medium and experimental conditions	Comments and results
Ralph and Catcheside (1999)	<ul style="list-style-type: none"> • Two HAs from Morwell brown coal: the first one was soluble between pH 3 and 4.5, and the second one, soluble below pH 6. The first one was ultrafiltered through a 30-kDa membrane and the second one, methylated and ultrafiltered through a 10-kDa membrane. • LiP from the white-rot fungus <i>Phanerochaete chrysosporium</i> • Into 100-mL conical flasks, containing 20 mL buffer (10 mmol/L sodium tartrate, pH 4.5) with 0.2 mg HA (and sometimes, NaVO₃), two separate solutions were added dropwise and concurrently over 4 h: 5 mL buffer containing 30 μmol H₂O₂ and 5 mL buffer containing 14.9 μmol veratryl alcohol and 8.6 units LiP. The flasks were stirred in darkness at 25 °C during this 4-h period and for a further 20 h. Some flasks were subjected to oxygen sparging. 	<ul style="list-style-type: none"> • For the non-methylated HA, the reduction of absorbances at 280 and 400 nm were of around 40% in LiP+H₂O₂+veratryl alcohol and of 30% in LiP+H₂O₂+veratryl alcohol+oxygen. The combinations of LiP+H₂O₂ and LiP+H₂O₂+veratryl alcohol+NaVO₃ did not cause considerable decolorization (1 – 3%). Within the experimental error, the modal apparent molecular mass (determined by gel permeation chromatography) and the ratio of absorbances at 280 and 400 nm did not change in any reaction. • For the methylated HA, reduction of absorbances were of 6% at 280 nm and of 7% at 400 nm in LiP+H₂O₂ and of 27% at 280 nm and of 37% at 400 nm in LiP+H₂O₂+veratryl alcohol. The decrease of the modal mass and the increase of the absorbance ratio were only important for the second combination (23.5% reduction of mass and 15% rise of the ratio). Combinations of LiP+H₂O₂+veratryl alcohol+oxygen and of LiP+H₂O₂+veratryl alcohol+NaVO₃ were not tested. • It was stated that NaVO₃ inhibited the action of LiP, that the methylated HA was more easily degraded by LiP because it was more similar to the lignin than the non-methylated HA, and that this non-methylated species inhibited the LiP-catalysed oxidation of veratryl alcohol.

Autor	Fungi, medium and experimental conditions	Comments and results
Claus and Filip (1998)	<ul style="list-style-type: none"> • HA from groundwater (Fuhrberg, Germany), HA and FA from bog lake (Hohlohsee, Germany) and HS from the Saint Mary's River (Georgia, USA) • Lac from <i>Polyporus versicolor</i> strain DSM 3086 • 0.9 mL of a solution containing 0.5 g/L lake HA in 100 mmol/L phosphate buffer (pH 5.9) were mixed with 0.1 mL of a Lac preparation (12 µg/mL protein and 2500 nkat/mg specific activity). The effect of adding 0 – 4 mmol/L of 1-hydroxybenzotriazole (a redox mediator) was tested. The mixture was incubated for 7 days at 30°C • When determined the optimum concentration of redox mediator, the experiment was repeated with FA from lake water, HS from river and HA from groundwater 	<ul style="list-style-type: none"> • The initial idea was to use Lac from <i>C. cladosporioides</i> P8, but preliminary tests revealed that its enzymatic activity in media containing HA was lower than that of the Lac from <i>P. versicolor</i>. Furthermore, the activities of both enzymes were functions of the HA concentration and its origin. • Decolorization of HA from bog lake (determined through the absorbance at 465 nm) reached a maximum of 50.5% at 2 mmol/L redox mediator. • Decolorizations for FA from lake water, HS from river and HA from groundwater in liquid media containing 2 mmol/L redox mediator were of 39, 37 and 45%, respectively. The ratios of absorbances at 465 nm and 665 nm increased a 222% for lake FA, a 123% for river HS and a 167.5% for groundwater HA.
Gramss et al. (1999)	<ul style="list-style-type: none"> • HS from a black calcareous forest mull • Five oxidoreductase enzymes (catalase from bobine liver, glucose oxidase from <i>Aspergillus niger</i>, Lac from <i>Pyricularia oryzae</i>, horseradish peroxidase coupled with H₂O₂ and tyrosinase from <i>Agaricus bisporius</i>), one hydrolase (β-glucosidase from almonds) and two abiotic oxidants (hydrogen peroxide and manganese triacetate). • Serum bottles containing 4 mL of sterilized HS solutions (1 g/L HS in 0.1 mol/L citrate-phosphate buffer) amended with 1 mL enzyme/oxidant solution. Incubation at 24°C for 24 h. 	<ul style="list-style-type: none"> • Concentrations of the enzymes and oxidants were: catalase (1.4 g/L), glucose oxidase (1.2 g/L), Lac (2.0 g/L), peroxidase (0.22 g/L coupled with 1 g/L H₂O₂), tyrosinase (2.2 g/L), β-glucosidase (1.2 g/L), hydrogen peroxide (1.0 g/L) and manganese triacetate (0.5 g/L). • Tyrosinase (a MMO) and catalase did not reduce the absorbance of HS solutions at 340 nm. Glucose oxidase and Lac only gave decolorations of 2.9% and 3.4%, respectively. β-glucosidase caused a fall of 21.5% in the absorbance, but a 19.5% was due to precipitation by formation of enzyme-HS complexes. • Hydrogen peroxide, manganese triacetate (representative of MnP) and plant peroxidase achieved decolorizations of 9.3, 13.9 and 18.3%, respectively.

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Wunderwald et al. (2000)	<ul style="list-style-type: none"> • Fluorinated HA, synthesized by spontaneous oxidative polymerization of 3-fluorocatechol. It contained 50.4% C, 2.6% H, 13.6% F and 33.4% O. • MnP from the white-rot fungus <i>Nematoloma frowardii</i> b19 • 100-mL flasks containing 20 mL of the reaction mixture (50 mmol/L sodium malonate buffer, pH 4.5, 1 mmol/L MnCl₂, 0.6 mmol/L glutathione, 100 mg/L HA, 500 U/L MnP and H₂O₂ continuously generated by glucose/glucose oxidase from <i>Aspergillus niger</i>), incubated at 37 °C in the dark and stirred continuously for 75 h 	<ul style="list-style-type: none"> • In the same paper, it was found that the fungus <i>Nematoloma frowardii</i> b19 degraded the HA, and that its main excreted enzyme was MnP. • MnP was able of degrading the HA, because the unbounded fluorine in the medium was 0.01 mmol/L F₂ at the beginning of the reaction, quickly rose to around 0.18 mmol/L F₂ after 5 h, and then, increased slowly to 0.325 mmol/L F₂ after 75 h. • Gel permeation chromatography indicated that during the first 5 h incubation, about 80% of the HA was converted, but that the modal molecular mass of the residual HA was not changed. FA production was investigated as well: this fraction increased simultaneously with the HA conversion and its modal mass slightly rose. After 75 h the 100% of the HA had been degraded.
Zavarzina et al. (2004)	<ul style="list-style-type: none"> • HA from peat, soddy-podzolic soil and chernozem • Lac of the white-rot basidiomycete <i>Panus tigrinus</i> 8/18 • Incubation mixtures contained 0.4 mL HA (2 mg) and 0.02 mL Lac preparation (0.48 mg/mL) in 1 mL buffer (20 mmol/L sodium-acetate buffer, pH 5.0) at 30°C for 96 h 	<ul style="list-style-type: none"> • Absorbances at 280 nm decreased a 26.9% for peat HA and a 13% for soddy-podzolic HA, but increased a 11% for chernozem HA. The ratio of absorbances at 465 nm and at 650 nm always decreased: a 12% for peat HA, a 5% for soddy-podzolic HA and a 11% for chernozem HA. • Gel chromatography showed that the HA from chernozem was degraded (the relative content of its low molecular weight fraction increased, apparently in expense of high molecular weight fraction), whereas the HA from peat suffered polymerization (the content of its high molecular weight fraction increased as well as the average molecular weight of the low molecular weight fraction). For the HA from soddy-podzolic soil, the result was intermediate: the content of high molecular weight fraction decreased, but the average molecular weight of low molecular weight fraction increased • Further experiments on inhibition of Lac by HA probed that this existed, that it was of the competitive type and increased with increasing HA concentration.

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Zahmetkesh et al. (2016)	<ul style="list-style-type: none"> • Coal HA, containing a 3% small molecules (<0.5 kDa), a 27% medium-sized compounds (0.5 – 1 kDa) and a 70% large substances (1 – 6.5 kDa). • Lac from <i>Trametes versicolor</i>. • 150-mL flasks containing 50 mL of Lac solution at 500 U/L in malonate buffer (pH 4.5) and 2 mL HA solution (for a final concentration around 308 mg/L), incubated at 26°C and 130 rpm for 24 h. Occasionally, 1 mmol/L of Lac mediator was added. 	<ul style="list-style-type: none"> • Lac mediators were 1-hydroxy-benzotriazol hydrate, 2,2'-azino-bis-(3-ethylbenz-thiazoline-6-sulfonic acid) and violuric acid. Size exclusion chromatography was the only analytical technique used. • Reduction of chromatographic area was of 20.6% for Lac alone, of 64% for Lac with 2,2'-azino-bis-(3-ethylbenz-thiazoline-6-sulfonic acid), of 32% for Lac with 1-hydroxy-benzotriazol hydrate and of 19.8% for Lac with violuric acid. • Proportions of 4% small, 7% medium and 89% large molecules for Lac alone, of 2% small, 8% medium and 90% large compounds for Lac plus 2,2'-azino-bis-(3-ethylbenz-thiazoline-6-sulfonic acid), of 2% small, 32% medium and 66% large substances for Lac plus 1-hydroxy-benzotriazol and of 2%, 44% medium and 54% large molecules for Lac plus violuric acid were found.

Media where the reactions took place are described in these tables, except those of Kirk et al. (1978) and of Tien and Kirk (1988), in order to avoid an excessive size of the rows. They are as follows: for Kirk et al. (1978), the basal medium contained 0.2 g/L KH_2PO_4 , 0.05 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g/L CaCl_2 , 1 mL mineral solution and 0.5 mL vitamin solution. This mineral solution consisted of 1.5 g/L nitrilotriacetate, 3 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/L $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 1.0 g/L NaCl , 100 mg/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 100 mg/L CoSO_4 , 82 mg/L CaCl_2 , 100 mg/L ZnSO_4 , 10 mg/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 10 mg/L $\text{AlK}(\text{SO}_4)_2$, 10 mg/L H_3BO_3 , 10 mg/L NaMoO_4 , whereas the vitamin solution consisted of 2 mg/L biotin, 2 mg/L folic acid, 5 mg/L thiamine·HCl, 5 mg/L riboflavin, 10 mg/L pyridoxine·HCl, 0.1 mg/L cyanocobalamine, 5 mg/L nicotinic acid, 5 mg/L DL-calcium pantothenate, 5 mg/L *p*-aminobenzoic acid and 5 mg/L thiocctic acid. For Tien and Kirk (1988), the medium contained 1.68% glucose, 16.8 mmol/L 2,2-dimethylsuccinate (pH 4.2), 1.68 mg/L thiamin, 0.336 g/L ammonium tartrate, 0.67% veratryl alcohol, 100 mL spore suspension, 100 mL basal medium and 60 mL trace element solution. This basal medium consisted of 20 g/L KH_2PO_4 , 5 g/L MgSO_4 and 1 g/L CaCl_2 and 100 mL trace elements solution, whereas the components of the trace element solution were 3 g/L MgSO_4 , 0.5 g/L MnSO_4 , 1.0 g/L NaCl , 0.1 g/L

FeSO₄·7H₂O, 0.1 g/L COCl₂, 0.1 g/L ZnSO₄·7H₂O, 0.1 g/L CuSO₄, 10 mg/L AlK(SO₄)₂·12H₂O, 10 mg/L H₃BO₃, 10 mg/L Na₂MoO₄·2H₂O and 1.5 g/L nitrilotriacetate.

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Tien, M., Kirk, T.K., 1988. Lignin peroxidase of *Phanerochaete chrysosporium*. in: *Methods Enzymol.*, Vol. 161, Academic Press, pp. 238-249.