LEACHATES AND NATURAL ORGANIC MATTER. A REVIEW OF THEIR BIOTREATMENT USING FUNGI

Sergio Collado[a], Paula Oulego[a], Octavio Suárez-Iglesias[a], and Mario Díaz[a#].

[a]Department of Chemical and Environmental Engineering, University of Oviedo, c/ Julián Clavería s/n, E-33071, Oviedo, Spain.

Tfno.: (+34) 985 10 34 41

Fax: (+34) 985 10 34 34

Address correspondence to Mario Díaz, mariodiaz@uniovi.es.

ABSTRACT

Leachates have different concentrations of organic matter and levels of biodegradability, depending on the age of the landfill and they must be treated using appropriate techniques, such as fungal degradation, in order to protect the environment and water resources. Natural organic matter contains the same type of organic species as old and medium age leachates, but at lower concentrations. The present study compiles and assesses all the available literature on the biotreatment of these compounds, mainly humic acids, by fungi.

It was found that the efficiency of the fungal biodegradation of these wastewaters depends on the characteristics and concentration of the organic matter in the leachate, the microorganisms selected and whether they were immobilized or not, the nutrients present in the medium and their concentrations, the experimentation time, the temperature and the pH. The influence of the mode of inoculation has only been studied in natural organic matter, but similar effects are expected in the treatment of the leachates.

The interactions between these parameters are complex and the optimal conditions have to be determined by laboratory and pilot testing, employing multivariate statistical techniques and experimental design.

Keywords: Biodegradation; Enzyme; Fungi; Humic acids; Leachates; Natural organic matter.

1. INTRODUCTION

Landfill leachate is the polluted aqueous effluent from a landfill due to percolation of rain through it, the moisture in the deposited waste, surface water runoff

and biological degradation (Bodzek et al., 2006). The water dissolves and suspends organic and inorganic compounds from the decomposing waste deposits, acquiring high levels of Chemical Oxygen Demand (COD), 5-day Biological Oxygen Demand (BOD_5), Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), Total Nitrogen (TN), Total Kjeldahl Nitrogen (TKN), Ammonia Nitrogen (NH_4-N), heavy metals and toxicity (evaluated through inhibition of certain animal and vegetal species). Therefore, such effluent can severely harm the environment if it is discharged without previous treatment (Di Maria and Sisani, 2017).

There are several factors that affect the amount and composition of the leachate, although rainfall (related to the quantity) and age of the landfill (related to the quality of the stream) are the most reported ones (Renou et al., 2008). With regard to this second point, it has been shown that the buried waste goes through a four-stage degradation process: i) an aerobic stage, where the oxygen present in the recently deposited wastes is quickly consumed; ii) a fermentative one after the depletion of the oxygen, during which high amounts of carboxylic acids are generated; iii) a methanogenic phase, where the acids become methane and carbon dioxide; and iv) a final maturation stage, with low microbial activity and low production of methane (Kjeldsen et al., 2002; Ren & Yuan, 2015).

Young leachates are those which come from landfills less than 1 year old, where the aerobic and acidic stages prevail; the COD is above 15 g/L, carboxylic acids constitute more than 80% of the aqueous stream and the ratio between the biodegradable (as BOD_5) and the total organic matter (as COD) is higher than 0.5. Old leachates are those from facilities which are more than 5 years old, during the maturation phase, with COD below 3 g/L and mainly composed of a refractory mixture

of Humic Acids (HA) and Fulvic Acids (FA), denominated "Humic Substances" (HS). Their BOD_5 to COD ratio is lower than 0.1. Finally, medium-age leachates are generated in landfills of 1 to 5 years old, and therefore in the methanogenic phase, and display intermediate properties between those of the young and old leachates (Gao et al., 2015).

Due to their relatively high biodegradability, young leachates can be treated by biological processes in which bacteria predominate, such as activated sludge, nitrification-denitrification biotreatments or anaerobic fermentations (Kurniawan et al., 2010). However, for medium-age and old leachates, in which the proportion of refractory HS is higher, physicochemical process such as adsorption (Foo & Hameed, 2009), membrane filtrations (Bodzek et al., 2006; Kurniawan et al., 2006a), electrochemical techniques (Fernandes et al., 2015), chemical or photochemical oxidations (Kurniawan et al., 2006b), wet air oxidation (Anglada et al., 2011; Oulego et al., 2016) and combinations of them are preferred.

At this point, it should be noted that there is another pollutant that is closely related to leachates: Natural Organic Matter (NOM). Like leachates, this is a complex mixture of thousands of organic compounds found in water, highly variable, with low biodegradability and mainly composed of HS. What is more, the ways that the two pollutants are generated are similar: NOM is derived from decaying plant and animal matter, whereas leachates are formed from decomposing landfill wastes (Kosobucki and Buszewski, 2014). The removal of NOM is necessary, since its elimination allows the disinfection or desalination processes required to render waters potable to be carried out under milder conditions (Matilainen et al, 2011). It seems reasonable to propose that many

findings and conclusions reached for NOM treatments can be extrapolated to leachates and vice versa.

In recent years, fungal-based processes have been applied to the treatment of leachates and NOM. Fungi are a kingdom of heterotrophic eukaryotes that grow individually (yeasts) or forming hyphae and mycelium (moulds), reproduce sexually or asexually through spores (Sankaran et al., 2010) and mineralize HS (Grinhut et al., 2007). Ascomycetes and basidiomycetes are the most widely used subkingdoms of fungi in bioremediation (Tortella et al., 2005). In particular, the wood-degrading white-rot fungi, which decompose the lignin polymers by the secretion of ligninolytic enzymes: Lignin Peroxidase (LiP), Manganese Peroxidase (MnP) and Laccase (Lac), are frequently used (Yadav & Yadav, 2015).

It is worth stressing that these enzymes also degrade recalcitrant pollutants, which are commonly treated by physicochemical techniques in textile, pulp and paper, and olive mills and in the petrochemical and brewery industries (Garg & Modi, 1999; Sen et al., 2016; Singh, 2006), since these contaminants display certain similarity with lignin. Lac directly utilizes molecular oxygen as oxidant, whereas LiP and MnP employ veratryl alcohol and Mn(III), respectively, which are generated by oxidation with H_2O_2 of substances previously produced by other non-ligninolytic enzymes secreted by the fungi themselves (Ikehata et al., 2004; Pointing, 2001).

This paper reviews all the studies about fungal bioremediation of leachates published to date, using both the fungal cells and also the enzymes isolated from them alone. This is an in-depth study, which completes the previously existing ones by Gotvajn and Pavko (2015) and Gosh and Thakur (2017), while adding more bibliographic references. It is noteworthy that the present work also evaluates the different variables

that influence the reactions, such as the type of leachate and microorganisms, the presence or absence of fungal immobilization, medium nature and nutrients, temperature and pH.

Additionally, taking into account the above-mentioned similarities between leachates and NOM-containing waters, references referring to the removal of NOM by fungal treatments are also revised and discussed in this work (Bhatnagar & Sillanpää, 2017; Oulego et al., 2016; Reemtsma, 2009). (Fig. 1)

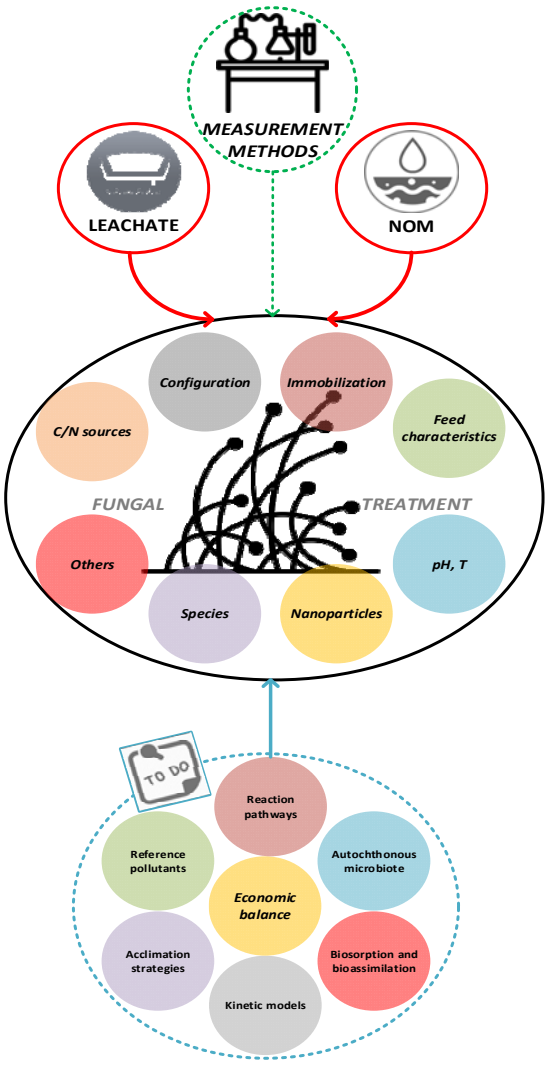

Figure 1. Scheme of this study, analysing the biodegradation of leachates and natural organic matter.

2. MEASUREMENT METHODS

Before beginning with the revision, the way the efficiency of the fungal treatment is measured should be discussed. In this regard, COD, BOD_5, TOC, NH_4-N, TN, TKN, colour, aromatic bonds and HS are the most commonly used parameters for the quantification of degradation efficiency. The first five of them are usually determined by standard methods (APHA, 2005); while the decolorization, or bleaching, is evaluated by means of values of absorbance in the visible range (Bardi et al., 2017b; Brito, 2013; Gao et al., 2004; Kim et al., 2003; Lee, 2005; Rojek et al., 2004; Solarska et al., 2009; Tigini et al., 2014; Tigini et al., 2013), the ADMI tristimulus filter (Saetang, 2009; Saetang & Babel, 2010a; Saetang & Babel, 2009; Saetang & Babel, 2010b) or the platinum-cobalt method (Ghosh et al., 2014). With respect to the loss of aromaticity, ultraviolet absorbance measurement is employed (Lee, 2005), as it is for HS quantification (Gao et al., 2004). For the latter, a modified Lowry method is also accepted as a measurement method (Brito, 2013; Brito et al., 2012).

The evolution of the leachate components during the biotreatment reaction has also been followed by the use of more complex measurement methods, such as size exclusion chromatography, from the fingerprints and reduction in the area of chromatograms with time (Thomson et al., 2004). This technique also helps to determine which leachate fractions are more easily attacked and the changes in the modal mass at the end of the biotreatment (Lee, 2005; Solarska et al., 2009). Likewise, ultrafiltration may also provide information about the evolution of molecular weight distributions for the leachate, although these are less detailed than those obtained from size exclusion chromatography (Wichitsathian et al., 2004). The evolution of toxicity during fungal treatment is another important parameter to monitor; the measurement methods for this variable include

inhibition of the bioluminescence of *Vibrio fischeri* (Amaral et al, 2017b; Reis et al, 2017; Kalcikova et al, 2014; Ellouze et al, 2008), the determination of the germination index using *Lepidium sativum* (Spina et al, 2018; Tigini et al, 2014, 2013; Ellouze et al, 2007), *Cucumis sativus* (Tigini et al, 2014), *Sinapis alba* (Kalcikova et al, 2014) or *Zea mays* (Awasthi et al, 2017) seeds, the inhibition of algal growth using *Pseudokirchneriella subcapitata* (Tigini et al, 2013) or *Raphidocelis subcapitat*a (Spina et al, 2018), the mobility inhibition of the fresh water crustacean *Ceriodaphnia dubia* (Tigini et al 2014) or by means of alkaline single-cell gel electrophoresis (Comet assay) (Gosh et al, 2014).

With respect to the monitoring of the biomass, it is opportune to point out that there is no direct link between fungal growth and biodegradation. Obviously, biodegradation is accompanied by a rise in the biomass weight (Bardi et al., 2017b; Kim et al., 2003; Rojek et al., 2004) or cell concentration (Brito, 2013; Brito et al., 2012). Nevertheless, those conditions which favour higher growth rates do not always result in better biodegradation and higher enzyme activity (Bardi et al., 2017b; Lee, 2005; Saetang & Babel, 2009).

It is well known that fungi excrete extracellular enzymes that decompose complex nutrients into simpler substances that can easily be absorbed through the cell wall. As mentioned earlier, notable among these for their scope of application and interest are the ligninolytic enzymes, (LiP, MnP and Lac), which directly attack the lignin (or lignin-like) polymers, including the HS (Collado et al., 2018). The enzymatic activities (U) of these are estimated from the oxidation rates of certain substrates. In the case of Lac enzyme, these substrates include 2,2'-azinobis(3-ethylbenzthiazoline-6-sulphonate) (Ellouze et al., 2008; Ellouze et al., 2009; Kalčíková et al., 2014; Saetang, 2009; Saetang & Babel, 2010a; Tigini et al., 2013), guaiacol (Lee, 2005; Solarska et al., 2009) or syringaldazine

(Abdullah et al., 2013). In the case of MnP activity, this is determined using 2,6-dimethyoxiphenol (Kalčíková et al., 2014; Lee, 2005; Solarska et al., 2009), vanillylacetone (Ellouze et al., 2008; Ellouze et al., 2009), phenol red (Saetang, 2009; Saetang & Babel, 2010a), guaiacol (Abdullah et al., 2013) or 3-dimethylaminobenzoic acid/ 3-methyl-2-benzothiazolinone hydrazone hydrochloride (Bardi et al., 2017a; Bardi et al., 2017b; Tigini et al., 2013). Finally, veratryl alcohol (Abdullah et al., 2013; Ellouze et al., 2008; Ellouze et al., 2009; Lee, 2005; Solarska et al., 2009) or azure B (Saetang, 2009; Saetang & Babel, 2010a) are the most common substrates for the quantification of LiP activity. It is important to note here that the presence of leachate in the medium during the tests can either increase or decrease the enzymatic activity with respect to a reference experiment with only the substrate. This effect of the presence of leachate depends also on the fungus selected (Ellouze et al., 2009).

Figure 2 shows examples of this kind of time-dependence between these variables.

Several authors have pointed out that fungi are also able to remove some pollutants by adsorption onto the biomass (Al Mamun et al., 2011; Vaverková et al., 2018; Zhou & Banks, 1993), and therefore, the reductions in COD, BOD_5, TOC, NH_4-N, TN, TKN, colour, aromaticity and HS are not only due to the fungal bio-assimilation, but also to a biosorption process.

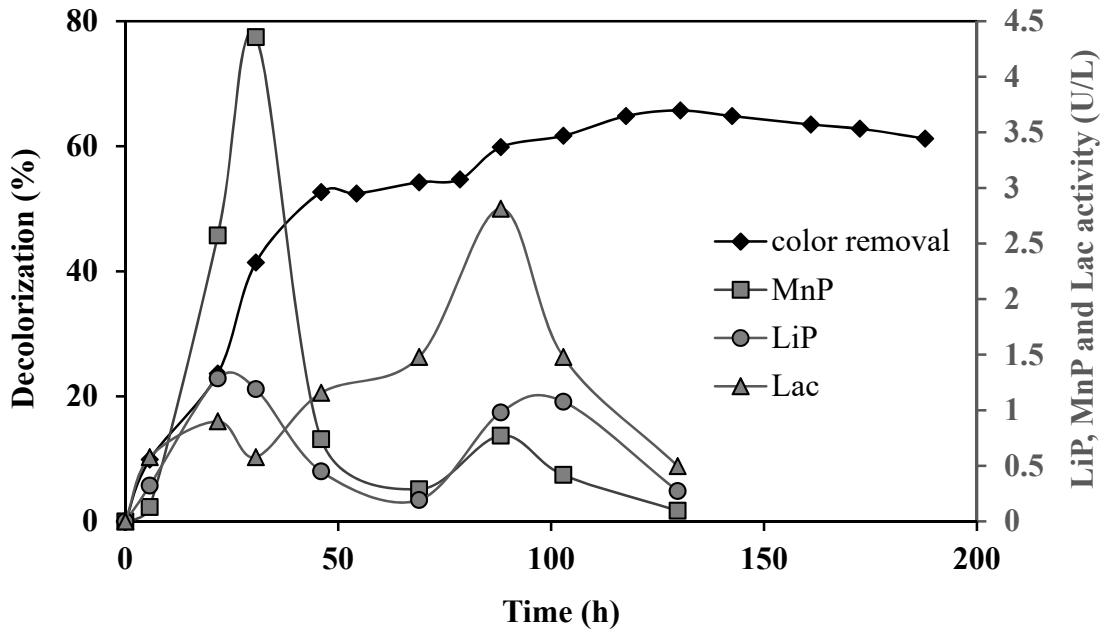

Figure 2. Time-evolution of some variables during fungal biodegradation: (A) Colour removal and enzymatic activities for the leachate of Solarksa et al. (2009) treated with *B. adusta* (B) COD, BOD5, colour removal and biomass growth for the leachate reported by Saetang and Babel (2009) with 600 mg/L glucose and 5-times diluted, treated with *T. versicolor* immobilized for 15 days.

3. NATURAL ORGANIC MATTER (NOM)

As noted above, NOM is considered to be a complex, heterogeneous mixture of organic compounds, with HS being the major component. (Bhatnagar & Sillanpää, 2017; Sillanpää, 2015). As can be seen, there are many parallels between NOM and leachate and indeed, the former could even be considered as a diluted leachate arising from a natural source.

Nevertheless, knowledge about NOM removal by fungi is scarce. After a deep bibliographic review, only four studies dealing with this topic have been found, and all

utilize NOM from Hope Valley Reservoir (South Australia) concentrated by ion exchange. Table 1 provides an overview of them (for more detailed information, see Supporting information, Tables S1 and S2).

3.1. Fungal species

As can be seen in Table 1, *Phanerochaete chrysosporium* ATCC 34541 and *Trametes versicolor* ATCC 7731 were the fungi most frequently employed for NOM removal. Lee (2005) compared *T. versicolor* with three yeasts (*Saccharomyces* species 1, 2 and 3) and two *P. chrysosporium* strains (ATCC 34541 and ATCC 24725) in a modified Waksman medium with a NOM concentration of 100 mgC/ L in a 5-day experiment. Of the white-rot fungi, *T. versicolor* achieved the highest colour removal (59%), whereas the two strains of *P. chrysosporium* displayed comparable colour reduction (37%). *Saccharomyces* sp. 1 and 3 seemed to have little potential for the removal of NOM (10 and 22%, respectively). On the other hand, *Saccharomyces* sp. 2 gave a similarly high level of colour reduction to *T. versicolor*. The specific removal values differed markedly, however, being 0.055 compared to 0.089 mg NOM/mg biomass, respectively. The colour removal by the *Saccharomyces* species was attributed predominantly to adsorption, as indicated by the deep brown colouration of the biomass for all species. The yeast removed little UV-absorbing NOM and there was only a small reduction in high molecular weight range compounds and no formation of lower molecular weight materials. Consequently, enzymatic activities were only measured for *T. versicolor* and *P. chrysosporium*, showing that they mainly secreted Lac and LiP, respectively, and that Lac activities in *T. versicolor* ATCC 7731 were higher than those of LiP in *P. chrysosporium* ATCC 34541 and ATCC 24725. Moreover, the low concentration of MnP in the ATCC 3451 (compared with those of LiP) could explain why

Rojek et al. (2004) did not see improvement in decolourisation when adding Mn(II) to the same fungus (i.e., because MnP did not play a significant role in the reaction). Solarska et al. (2009) also compared *T. versicolor* ATCC 7731 with *Bjerkandera adusta, Trametes* sp. and *Polyporus* sp. in a medium without nutrients. All four fungi decolorized NOM, with *B. adusta* attaining the greatest reduction, of 65%. Lac was the most active enzyme excreted. However, its enzymatic activity did not correlate with the degree of decolourisation. On the contrary, even when the activities of Lac, MnP and LiP for *B. adusta* were the lowest, this microorganism achieved the highest colour removal.

3.2. NOM concentration and nature

Not only the fungal species, but also the characteristics of the NOM play a key role in the efficiency of their fungal biotreatment activity. So, Lee (2015) reported that NOM removal (mg removed) by *Trametes versicolor* increased linearly with NOM concentration up to 600 mg C/L, above which point it decreased markedly. These authors also observed that *Phanerochaete chrysosporium* seemed to preferentially remove NOM with a high proportion of hydrophobic compounds, mainly HA and FA. Since the latter are responsible for the high levels of colour, aromaticity and the high SUVA value, these characteristics were greatly reduced during treatment (Lee, 2005).

3.3. Additional C and/or N sources

Regarding the effect of the availability of nutrients, the NOM bleaching caused by *Phanerochaete chrysosporium* and *Trametes versicolor* is more marked if the amount of N in the medium is increased (Lee, 2005; Rojek et al., 2004). However, for the same initial N concentration, increasing amounts of glucose can increase (Rojek et al., 2004) or decrease (Lee, 2005) the decolourisation rate of the NOM. This suggests that the NOM

bleaching caused by the fungi is not only a function of the C:N ratio, but also of the total C and N concentrations. For *P. chrysosporium*, Rojek et al. (2004) reported that the amounts of C and N can also modify the effect of the NOM concentration on its bleaching rate. Thus, the degree of decolorization decreased with increased NOM content in a low glucose medium, but remained approximately constant in media containing sufficient glucose.

3.4. Other studies

For *T. versicolor*, Lee (2005) also tested the influence of other parameters such as temperature and inoculation mode. Regarding the former, their results are not very conclusive: after 3 days, NOM bleaching by *T. versicolor* was higher at 36ºC than at 30ºC, but after 9 days, the trend reversed and what happened was just the opposite. In any case, NOM biosorption was favoured at 36ºC. When it comes to inoculation mode, comparison of NOM removal using inoculation by spores and by plugs was undertaken. Although the degree of NOM removal (measured as decolourisation) was 8% greater when the spore suspension inoculum was used, the plug inoculation method was much simpler. Furthermore, plug inoculation led to higher enzyme activity than spore inoculation, even though there was a longer lag phase for the enzyme secretion, and thus provides better potential for the biodegradation of NOM.

It is important to emphasise that, with the exception of Thomson et al. (2004), who did not specify whether or not there is biosorption in their experiments, most of the authors bore the adsorption of NOM by the fungal hyphae in mind, although its contribution to the total NOM removal was not quantified exactly. Only in one of the many experiments performed by Lee (2005), where wheat bran was added to the medium, was the adsorption due to this substance exactly determined and subtracted from the

global bleaching. Among the aforementioned authors, Rojeck et al. (2004) observed that the reduction in the pH from 5.8 to 3 and the increase in the ionic strength from 0 to 50 g/L NaCl reduced biomass production but improved the decolourisation per unit of biomass. This led them to suspect that most of the NOM removal by *P. chrysosporium* was due to biosorption, a speculation which was later confirmed by experiments with fungal pellets. Lee (2005) also confirmed the important role of bioadsorption in NOM bleaching: NOM adsorption was responsible for the deep brown colorization of the biomass of *P. chrysosporium*, whereas biomass pellets formed from *T. versicolor* were creamy brown in colour, which indicated no NOM adsorption onto the biomass in this latter case. These authors also noticed that adsorption was favoured at the highest glucose content, whereas addition of Tween 20 reduced the Lac activities and the decolourisation (although in NOM-free media, the surfactant increased the activity of Lac). The addition of both Tween 20 and wheat bran to a high-glucose modified Waksman medium did not improve the biodegradation, but only the biosorption of NOM. In all these cases, and despite adsorption, high bleaching was related to high enzymatic activity, except when the NOM concentration was varied. In that case, the relationship was between the enzyme activity and the "concentration of NOM removed". The latter was defined as the product of the decolourization percentage and the initial NOM concentration (Lee, 2005). Furthermore, the researcher demonstrated, by filtering an extracellular culture fluid and adding it to a mixture containing only NOM and a buffer, that these enzymes, when isolated, were capable of degrading NOM alone. The pH and the temperature effects on bleaching followed typical enzymatic behaviour, with the presence of an optimum point. Thus, an increase in these parameters enhances biodegradation, but beyond certain values they cause the denaturation of the enzyme (Lee, 2005).

Table 1. Fungal decolorization of NOM (for a more detailed description, see tables S1 and S2)

Ref.	Conditions	Measurements
Thomson et al.(2004)	*Phanerochaete chrysosporium* ATCC 34541	• Decrease in the chromatographic area and molar mass distribution.
Rojek et al.(2004)	*Phanerochaete chrysosporium* ATCC 34541	• Effect of N and C sources and pH and NaCl on decolorization, glucose consumption and biomass growth. • Influence of the adsorption on the results.
[Lee (2005)	NOM with 49% of very hydrophobic acids, 51% or 69% *Phanerochaete chrysosporium* strains ATCC 34541 and ATCC 24725, *Trametes versicolor* ATCC 7731 and three *Saccharomyces* species. Filtered extracellular culture fluid, enriched in Lac	• Decolorization, aromatic levels, glucose consumption, biomass weight and molar mass distribution for the three types of NOM treated by *P. chrysosporium* ATCC 34541 • Decolorization, glucose consumption and biomass weight for the NOM with 69% hydrophobic acids treated by *P. chrysosporium* ATCC 34541 in two media (a high-nitrogen one and a low-nitrogen one) • Decolorization, aromatic levels, glucose consumption, biomass weight and molar mass distribution for the NOM with 69% hydrophobic acids treated by the six fungi. • Enzymatic activities for *P. chrysosporium* and *T. versicolor*.

Ref.	Conditions	Measurements
		• Effect of temperature, inoculation mode (spores or agar plugs) and glucose present on decolorization, aromatic levels, glucose consumption, pH, biomass weight and enzymatic activities for the NOM with 69% hydrophobic acids treated by *Trametes versicolor* ATCC 7731. • Effect of NOM concentration and presence of Tween 20 or wheat bran on decolorization, aromatic levels, glucose consumption, biomass weight and enzymatic activities for the NOM with 69% hydrophobic acids treated by *Trametes versicolor* ATCC 7731. • Effect of temperature and pH on decolorization and aromatic levels for the NOM with 69% hydrophobic acids treated by enzymes
Solarska et al.(2009)	*Trametes* sp, *Polyporus* sp., *Trametes versicolor* ATCC 7731 and *Bjerkandera adusta*	• Time-dependence of decolorization, pH, enzymatic activities for the four fungi. Molar mass distribution after the incubation. • Influence of the adsorption in the results.

1

2

3

4 **4. LEACHATES**

Turning now to the treatment of leachate by fungi, one finds that the available literature on this topic is more abundant. Table 2 provides an overview of the main articles, whereas Table S3 can be consulted in order to obtain a more detailed description of these.

4.1. Fungal species

As mentioned above, the most favourable microorganisms for biological treatment are various species of white-rot fungi belonging to the class Basidiomycetes. This is because of their high growth rate and also their lignin biodegradation activity (Collado et al, 2018). Papers dealing with the comparison of two or more fungi to treat leachates have become numerous. The most frequently used genera are *Aspergillus* (Faco and Santaella, 2002;) *Trametes* (Saetang, 2009; Saetang and Babel, 2009, 2010a,b; Wan Razarinah et al, 2015) and *Phanerochaete* (Gao et al, 2004; Gosh et al, 2014; Hu et al, 2016, 2017), although studies using others, such as *Ganoderma* (Abdullah et al, 2013; Wan Razarinah et al, 2014), *Lentinus* (Ellouze et al, 2008,2009), *Dichomitus* (Kalcikova et al, 2014) or *Flavodon* (Saetang, 2009; Saetang and Babel, 2010b), as examples, are also reported in the bibliography.

Among the available studies, that carried out by Tigini et al (2014) should be highlighted. These authors identified the autochthonous mycoflora of a raw leachate and an effluent coming from a traditional wastewater treatment plant. Ascomycetes were the dominant fraction (81% and 61%, for leachate and effluent respectively), followed by basidiomycetes (19% and 39%, respectively). Additionally, a decolourisation screening was set up with autochthonous fungi from both samples in the presence or absence of glucose. Eleven fungi (basidiomycetes and ascomycetes) achieved up to 38% decolourisation yields after 20 h, and thus were shown to be promising fungi for the

bioremediation of leachates. Some isolates belonging to the genera *Aspergillus* (*Aspergillus fumigatus* MUT 4050, *Aspergillus tubingensis* MUT 1288, *Aspergillus sydowii* MUT 1290), *Arthrinium* (*Arthrinium sphaerospermum* MUT 777), *Penicillium* (*Penicillium brevicompactum* MUT 793, *Penicillium corylophilum* MUT 784), *Pseudallescheria* (*Pseudallescheria boydii* MUT 1269, MUT 721), and some basidiomycetes (*Bjerkandera adusta* MUT 765, *Phanerochaete sanguinea* MUT 1284, *Flammulina velutipes* MUT 1275) emerged as the most promising strains.

In a later work, Spina et al. (2018) also compared five autochthonous fungi (*Penicillium brevicompactum* MUT 793, *Pseudallescheria boydii* MUT 721, *P. boydii* MUT 1269, *Phanerochaete sanguinea* MUT 1284 and *Flammulina velutipes* MUT 1275) with five allochthonous fungal strains (*Pleurotus ostreatus* MUT 2976, *Porostereum spadiceum* MUT 1585, *Trametes pubescens* MUT 2400, *Bjerkandera adusta* MUT 3060 and *B. adusta* MUT 2295) in terms of leachate decolourisation, enzymatic activity (laccases and peroxidases), biomass growth and ecotoxicity removal. The best decolourisation (60%) was achieved by *P. spadiceum* MUT 1585, *P. boydii* MUT 721 and MUT 1269, but in the two last cases, biosorption was suspected. Results revealed that robust and versatile strains coming from well-characterized collections of microorganisms can obtain excellent results comparable with and even exceeding the bioremediation yields of strains already adapted to pollutants.

Ellouze et al (2008) evaluated the biological process using selected strains of *Trametes trogii*, *Phanerochaete chrysosporium*, *Lentinus tigrinus* and *Aspergillus niger* for the treatment of a young leachate. When the leachate underwent a two-fold dilution, COD removal efficiencies for *P. chrysosporium*, *T. trogii* and *L. tigrinus* were of 68, 79 and 90%, respectively, being accompanied with important levels of enzyme secretion and

a high reduction in the effluent toxicity. *A. niger* was shown to be the only one able to tolerate the undiluted leachate. However, this species was inefficient in removing phenols and hydrocarbons and consequently, toxicity abatement was very low.

4.2.- Bioreactor configuration

Another difference between the studies on leachates and on NOM is the scale of the tests and the state of the microorganisms. As previously reported, all the NOM experiments with fungi were done in flasks, whereas in this section, as well as flasks (Awasthi et al., 2017; Bardi et al., 2017b; Ellouze et al., 2008; Ellouze et al., 2009; Hu et al., 2017; Hu et al., 2016; Kalčíková et al., 2014; Saetang, 2009; Saetang & Babel, 2010a; Saetang & Babel, 2010b; Tigini et al., 2014; Tigini et al., 2013; Wan Razarinah et al., 2014; Wan Razarinah et al., 2015), other reactor configurations were employed, such as fixed bed (Faco and Santaella, 2002; Gao et al, 2004; Saetang, 2009; Saetang and Babel, 2009; Abdullah et al, 2013; Bardi et al. 2017a) or membrane bioreactors (Wichitsathian et al, 2006; Brito et al, 2012; Brito, 2013; Amaral et al, 2017ab; Reis et al, 2017). To our knowledge, there are no studies dealing with the biodegradation of leachate by a fungus, comparing the results obtained with different reactor configurations. It must be taken into account that by using small scale laboratory batch equipment, such as Erlenmeyer flasks, backmixing does not occur and the effect of the reaction rate may be isolated and examined alone. However, real hydrodynamic flow in large industrial equipment shows a high degree of backmixing, mainly due to the turbulence generated during the injection of the oxygen or air (Collado et al, 2013). Therefore, the choice of a batch operation mode for experimentation, rather than the typical continuous industrial operation with perfect mixing, involves a different evolution of the concentration of the intermediates during

biodegradation. Therefore, it would be very interesting to analyse the nature of any divergences that might appear due to backmixing in future research.

In experiments carried out in Erlenmeyer flasks, the duration of the experiments can range from 2 to 28 days, but the most common experimental times are between 4 and 10 hours (see table S3). For continuous bioreactors, typical hydraulic residence times for fixed bed reactors were around 3-4 days, whereas this parameter decreases to 1-2 days for the membrane bioreactor, probably due to the higher fungi concentration in the latter. (Table S3)

In all cases, it can be seen, as expected, that the longer the experimentation time, the greater the removal of pollutants. Obviously, this is because when the contact time is longer, the biomass growth is higher and the acclimatization better (Brito, 2013). Sometimes, the removal is not complete and tends to a plateau (Ellouze et al., 2008; Gao et al., 2004; Saetang & Babel, 2010a; Saetang & Babel, 2009; Saetang & Babel, 2010b). In other cases, authors have reported that it reaches a maximum and then declines (Spina et al., 2018; Ghosh et al., 2014; Kalčíková et al., 2014; Tigini et al., 2013) due to production and accumulation of nitrates, nitrites, orthophosphates (Facó & Santaella, 2002), ammonium (Wan Razarinah et al., 2014; Wan Razarinah et al., 2015), organics after cell death (Brito, 2013), toxic substances (Tigini et al., 2013) or coloured metabolites in the medium, all of which may act as inhibitors. In the experiments of Wichitsathian et al. (2004) the trend was only clear after implementing ammonia stripping (if this was not done, COD removal rose from 16 h to 24 h, but TKN removal fell slightly).

4.3. Leachate concentration and nature

Another aspect to be taken into account is the high variability in the composition of leachate used in these investigations. For example, Ellouze et al. (2008), Saetang and Babel (2010a, 2009), Abdullah et al. (2013), Tigini et al. (2014) and Wan Razarinah et al. (2014, 2015) employed young leachates, that is to say, streams with a BOD_5/COD ratio higher than 0.5. On the other hand, Kim et al. (2003), Gao et al. (2004), Brito (2013) Brito et al. (2012), Hu et al. (2017, 2016), Amaral et al. (2017a) and Bardi et al. (2017a,b) used mature leachates, with BOD_5/COD ratios lower than 0.1. Finally, Wichitsathian et al. (2004), Saetang (2009) and Saetang and Babel (2010b) selected a medium-age leachate for the experimentation, with $0.1<BOD_5/COD<0.5$. What is more, Tigini et al. (2013), Gosh et al. (2014), Awasthi et al.(2017), Amaral et al.(2017b), Reis et al.(2017) and Faco and Santaella (2002) did not give enough data to calculate this ratio, although the latter claimed that their leachate was in an advanced maturation stage, which agrees with a COD lower or around 3000 mg/L. Based simply on the COD criteria cited in the introduction, the leachates employed by Tigini et al. (2013), Awasthi et al. (2017) and Reis et al. (2017) should be classified as mature, while those of Gosh et al.(2014) and Amaral et al.(2017b) would be defined as medium-aged. However, it should be taken into account that this rule is not very reliable: some of the compiled papers report leachates with COD of around 3000 mg/L but with a value for the BOD_5/COD considerably higher than 0.1 (Kalčíková et al., 2014; Saetang, 2009) or even higher than 0.5 (Abdullah et al., 2013). Obviously, these high inconsistencies in the initial compositions of the leachates make it difficult to compare results from different papers and draw general conclusions about the fungal biotreatment of leachates. Only Kalcikova et al. (2014) compared the fungal biotreatment with *Dichomitus squalens* of two leachates with different values of BOD_5/COD: 0.33 (medium-aged) and 0.02 (mature). This fungus was able to grow in the mature leachate, utilizing the organic matter present as a source of carbon, achieving

DOC and COD removals of 60%, and decreasing leachate toxicity. Nevertheless, its growth was halted in medium-aged leachate, suggesting the presence of inhibiting components affecting the fungus in these less mature substrates. The authors proposed that such toxicants are probably degraded during waste stabilization in closed landfills, and are therefore not present in mature leachates.

When the initial toxicity of the leachate is too high, the stream is commonly diluted before treatment in order to improve the efficiency of the fungus. The dilutions employed differ between articles, but 50% (v/v) is the most prevalent (Abdullah et al., 2013; Awasthi et al., 2017; Gao et al., 2004; Kalčíková et al., 2014; Saetang & Babel, 2009). More specifically, Ellouze et al. (2008) evaluated the biodegradation by several fungi of a young leachate at different dilutions: 10, 30, 50, 70 and 100% leachate. The biological detoxification capacity of *Phanerochaete chrysosporium*, *Trametes trogii* and *Lentinus tigrinus* was very efficient in the case of 50% diluted leachate, with important COD reductions (68.8, 79.8 and 90.6%, respectively) and was accompanied by important levels of enzyme secretion for each fungus. However, on using concentrations of leachate exceeding 50%, mycelial growth was inhibited and no enzyme activity was detected. *Aspergillus niger* was found to be a very different case and demonstrated the ability to tolerate raw leachate. Indeed, the COD was greatly reduced (71%) with non-diluted leachate and ammoniacal nitrogen decreased by 80%. However, the strain seemed to be ineffective in eliminating phenols and hydrocarbons in the leachate. Although the results are rather difficult to interpret, because removal values have to be obtained from a poor-quality, low resolution figure and important COD reductions and biomass growths for all the proportions of leachates were observed, it seems that there was an optimal proportion (50%), for which the COD reduction was at its highest. This behaviour appears to be

reasonable since a higher dilution involves not only a reduction in the toxicity, but also in the quantity of nutrients available to the fungus.

Finally, Ellouze et al. (2008), Saetang and Babel (2010a) and Tigini et al. (2014) affirmed that there was a good correlation between the colour or COD removals in the young leachates and the ligninolytic enzyme activity, which in turn was dependent on the time at which the enzyme sample was extracted. However, in the work of Kalcikova et al. (2014) with old leachates, these removals seemed to correlate well with MnP, and not with Lac. Bardi et al.(2017a) proposed that this association depended on the co-substrate concentration, being evident when 2.5 g/L cellulose were added, but not when the concentration was 0.5, 1.0 or 5.0 g/L.

4.4. Additional C and/or N sources

Many authors consider that the presence of additional C sources promotes the removal of pollutant in the leachate by the fungus. In fact, the higher the additional C concentration, the higher the effectiveness of the fungal biotreatment is (Facó & Santaella, 2002; Saetang & Babel, 2010a; Saetang & Babel, 2009; Saetang & Babel, 2010b), although this tends to a plateau (Gao et al., 2004; Saetang, 2009). For example, Facó and Santaella (2002) reported COD removals by *A. niger* and *Cladosporium herbarum* for 2, 4 and 6 days of 34, 65 and 29%. However, when glucose was added (0.5 g/L) these values increased to 66, 84 and 63% for the same times. The addition of glucose also improved the growth of the native microbiota of the leachate (*Rhizopus* sp. and *Staphylococcus aureus*) and led to nitrification processes and to the accumulation of orthophosphates in the reactor. Similarly, the initial presence of 3 g/L of glucose during the treatment of an undiluted leachate by immobilized *Trametes versicolor* resulted in higher biomass growth, indicating that the growth of fungi is dependent on the co-

substrate (Saetang & Babel, 2010a; Saetang & Babel, 2009). The percentages of colour removal were approximately 58% and 12% after 12 h, respectively with and without glucose. BOD and COD removals also improved from 13% and 11% to 37% and 40% with glucose addition within 12 days at optimum conditions. Glucose also had a positive effect on production of Lac, MnP and LiP. The peak concentrations of LiP, MnP and Lac activities were found to be 384, 1,241, 2,534 U/L with glucose, indicating that the colour removal rates were proportional to the enzyme activity. Similar behaviour was reported for immobilized *Flavodon flavus* by Saetang & Babel (2009) and Saetang & Babel (2010b). These authors also observed that further experiments with concentrations of glucose higher than 3 g/L did not significantly improve the colour, BOD or COD removal. Similarly, when the glucose concentration was increased from 0.5 to 2 mg/L in a fixed bed reactor with immobilized *Phanerochaete chrysosporium*, the COD rose from 37 to 48%, but a subsequent increase to 5 g/L glucose did not involve a significant improvement (Gao et al 2004).

Nevertheless, it should be pointed out that these findings cannot be extrapolated to any genus. For example, after testing the biodegradation potential of the autochthonous mycoflora in a young leachate (*Aspergillus*, *Arthrinium*, *Penicillium*, *Pseudallescheria*), Tigini et al. (2014) observed that only in the cases of *Phanerochaete sanguinea* MUT 1284 and *Penicillium brevicompactum* MUT 793 was there a significant enhancement in the decolourisation rate in presence of 1 g/L glucose.

Some authors have investigated the effect of the type of carbon source on the treatment. Bardi et al (2007) studied enzyme production and COD removal by *Bjerkandera adusta* from old leachate as a function of the co-substrate used: cellulose, malt extract or glucose. The production of enzymes was positively correlated with

cellulose concentration up to 2.5 g/L, while at 5.0 g/L cellulose, the activity was clearly lower and there was no correlation between enzyme activity and COD reduction. The highest COD and soluble COD removals achieved were 63% and 53% with glucose and 54% and 51% with cellulose, respectively. These authors also carried out experiments with immobilized fungi and 1 g/L of the corresponding carbon source, but only MnP activity was measured. The highest activity was observed with malt extract, whereas cellulose gave the lowest. Saetang (2009) and Saetang and Babel (2010b) also tested the effect of 0-3g/L of glucose, corn starch or cassava on leachate degradation by immobilized *Trametes versicolor* or *Flavodon flavus*. These authors reported an optimum co-substrate concentration (glucose, corn starch and cassava) of 3 g/L with an optimum contact time of 10 days for both types of fungi. Addition of glucose, corn starch and cassava as co-substrate at optimum conditions removed 78, 74, and 66% of colour, respectively for *Trametes versicolor* and 73, 68, and 60%, respectively, for *Flavodon flavus*. The highest COD and BOD_5 removals were also observed with glucose as co-substrate, although the other C sources had a positive effect on these parameters as well. Gosh et al (2014) tested the roles of sucrose, dextrose, sodium citrate and sodium acetate (1% w/v) in leachate treatment using *Phanerochaete* sp ISTL01. Dextrose was found to be the best C source, causing 49% reduction in COD and 24% reduction in colour after 240 h of treatment.

Gosh et al (2014) also studied the effect of different N sources on *Phanerochaete* sp ISTL01 activity. These N sources were tryptone, yeast extract, sodium nitrate and sodium acetate (0.25 w/v). Yeast extract was the most suitable, with 43% reduction in COD and 22% reduction in colour after 240 h of treatment. In addition, they observed that there is an optimum N source concentration (yeast extract in this case) which produced a maximum in the COD and colour removals; higher concentrations were linked

to the inhibition of fungal activity by accumulation of high loads of NH_4-N in the medium. Similarly, Wichitsathian et al (2004) observed an improvement in the COD (from 63 to 76% after 24h) and TKN (from 28 to 86% for the same time) removals in an MBR loaded with a yeast sludge when the leachate was previously subjected to an ammonia stripping process. Due to the negative impact of the high ammoniacal nitrogen concentration on *Phanerochaete chrysosporium*, Gao et al (2004) tested three ways of removing it: air stripping at pH 11 and an air to water ratio of 3000, air stripping with pH 12 and an air to water ratio of 4000 and precipitation as magnesium ammonium phosphate, the last alternative being the most effective (91% NH_4-N removal). Additionally, the authors deliberately reduced the effectiveness of the pre-treatment process in order to investigate the influence of increasing amounts of NH_4-N on COD removal. As expected, this effect was negative. Thus, this removal decreased from 48% at 84 mg/L NH_4-N to 24% with 1025 mg/L NH_4-N. Ellouze et al (2009) carried out experiments dealing with the influence of NH_4-N on *Trametes trogii* and *Phanerochaete chrysosporium* in media without leachate. It was found that concentrations of NH_4Cl reaching 2g/L affected neither fungal growth nor enzyme secretion, but 5 g/L of ammonium chloride caused an inhibition effect on both fungal growth and enzyme production.

Finally, a means of reducing the consumption of the additional nutrient as well as improving the start-up of continuous bioreactors was proposed by Brito and Amaral, who started their membrane bioreactor with 20% leachate and 3 g/L of Sabouraud broth, then gradually reduced the dilution and, after introducing 100% leachate, decreased the broth content step by step, allowing a slow acclimatization of the *Saccharomyces cerevisiae* employed (Amaral et al., 2017a; Amaral et al., 2017b; Brito, 2013; Brito et al., 2012).

4.5. Fungal immobilization

The immobilization of the fungus is another issue that has not been investigated in NOM, and for this reason was not dealt with in the relevant section, but it has been addressed for leachates, in order to allow the recovery or reuse of the biomass for a hypothetical full-scale process. Additionally, immobilization leads to a higher resistance to shear stress, pH alterations and toxic substances, a higher surface area of the fungal biomass (which reduces the mass-transfer limitations, improving the contact between cells, oxygen and pollutants) and in some cases, to the enhancement of enzymatic production (Bardi et al., 2017a; Saetang, 2009; Saetang & Babel, 2009).

Passive immobilization (using the natural tendency of microorganisms to attach to surfaces, natural or synthetic, and grow on them) is the preferred mechanism. In these cases, the main materials used in fungal immobilization as the support include cinder (Gao et al., 2004), polyurethane foam (Bardi et al., 2017a; Bardi et al., 2017b; Saetang, 2009; Saetang & Babel, 2010a; Saetang & Babel, 2009; Saetang & Babel, 2010b; Spina et al., 2018) or Ecomat (Abdullah et al., 2013; Wan Razarinah et al., 2014; Wan Razarinah et al., 2015). Immobilization is carried out by putting the corresponding carrier in contact with the fungus in a growth medium (such as glucose-yeast-malt-peptone medium), usually for 4 days (Abdullah et al, 2013; Saetang, 2009; Saetang and Babel 2010a,b; Wan Razarinah et al 2015,2014), or 7 days (Bardi et al, 2017a,b). In this regard, the effect of the incubation period on the activity of immobilized fungus was only studied by Saetang and Babel (2009), comparing the behaviour of immobilized *Trametes versicolor* on polyurethane foam for 4 or 15 days during the biodegradation of a raw leachate. Results revealed that about 1-6 % higher colour removal efficiency was obtained on day 20 when fungus that had been immobilized for 15 days on PUF was used, compared with 4 days immobilization. However, when removal efficiencies were expressed per mg of biomass, it was found that with concentrated leachate, 4 days of immobilization and 3 g/L glucose

as co-substrate gave the highest removal, of 0.6 mg COD per mg of biomass and 0.45 mg BOD per mg of biomass (these values being slightly better than those obtained under the same conditions but with 15 day immobilization). Regarding the carrier size, this ranged from 0.4 cm^3 (Abdullah et al 2013) to 2 cm^3 (Bardi el al, 2017ab). For batch experiments, the number of units added per 100 ml of leachate were between 1.2 (Bardi et al 2017ab) and 10 (Saetang and Babel 2010b, 2009), whereas organic loads in continuous bioreactors vary from 5 to 25 mg BOD cm^{-2} L^{-1} d^{-1} (Saetang and Babel, 2009).

Only Hu et al (2016, 2017) used an active method of immobilization, based on the entrapment of *Phanerochaete chrysosporium* on Ca-alginate. These authors compared the TOC removal from an old leachate during its treatment with either the free or the immobilized fungus. Results revealed a slightly higher removal for the immobilized *P. chrysosporium* (53%) when compared to the free fungus (48%) (for an unspecified time).

4.6. Nanoparticles

In the aforementioned papers, Hu and co-workers also opened another interesting line of research: the loading of the immobilized fungus with nanoparticles, to enable leachate removal by either photocatalytic oxidation, biodegradation or biosorption. With this purpose, TiO_2 nanoparticles (Hu et al., 2016) or graphitic carbon nitride (Hu et al., 2017) were incorporated during the immobilization of *P. chrysosporium* on Ca-alginate. The authors then evaluated the effects of several operating parameters, such as initial chemical oxygen demand concentration, pH, temperature, and biosorbent dosage on leachate removal, in order to determine the optimal conditions. In both cases, the presence of nanoparticles considerably improved the leachate degradation in terms of TOC and NH_4^+ removal. For example, under the best conditions (200 mg/L leachate COD, 20 mg/L biosorbents and 37ºC, unspecified time) the following efficiencies were

reported: 40.15% TOC and 41.72% NH_4-N for free fungi, 54.97% TOC and 55.3% NH_4-N for fungi immobilized on Ca-alginate and 73.49% TOC and 72.09% NH_4-N for fungi immobilized together with nitrogen-doped TiO_2 nanoparticles on Ca-alginate (Hu et al, 2016). In this paper, the authors also reported that TOC removal reached a maximum at pH 6 before declining and NH_4-N removal peaked at pH 7. Subsequently, in an analogous series of experiments, Hu et al (2017) obtained TOC removals under lighting of 74.99% for the immobilized fungus with the graphitic carbon nitride, 44.83% for the immobilized fungus alone, 39.98% for the free fungus, 21.44% for immobilized graphitic carbon nitride and 24.95% for free graphitic carbon nitride. TOC removals without lighting were 32.18% for the immobilized fungus with graphitic carbon nitride, 42.01% for the immobilized fungus alone, 40.77% for the free fungus, 5.74% for the immobilized graphitic carbon nitride and 5.36% for the free graphitic carbon nitride. These results revealed that nanoparticles loaded on the hyphae played a key role in the improvement of photocatalytic ability for immobilized *P. chrysosporium*. The spatial retiform structure of immobilized *P. chrysosporium* allowed the organic matter in leachate to diffuse into its interior space and to make contact with the nanoparticles loaded on the hyphae surface. So, some refractory organic matter was degraded due to the photocatalytic activity of the nanoparticles, which resulted in the removal efficiency of immobilized *P. chrysosporium* being the highest. Meanwhile, the mechanical strength of immobilized *P. chrysosporium* was enormously enhanced by calcium alginate, thereby avoiding rupture and diffusion problems (Hu et al, 2017). To sum up, the leachate degradation was caused by a combination of biosorption, biodegradation and photodegradation.

4.7. pH and temperature

As with any biological process, pollutant removal in leachates by fungi increases with increasing temperature or pH, but beyond a certain value, it falls. For example, the TOC removal efficiency increased gradually at pH values ranging from 3.0 to 6.0, and then declined with a further increase in pH, during the biodegradation of an old landfill leachate by *Phanerochaete chrysosporium*, with values of 73.49% when using nitrogen-doped TiO_2 nanoparticles Ca-alginate immobilized *P. chrysosporium*, while with the free and the immobilized *P. chrysosporium* values of 40.15% and 54.97% were achieved, respectively (Hu et al 2016). Nevertheless, the optimal pH for NH_4^+ removal was slightly higher (7) than for TOC removal. At this pH value, removal efficiencies were 72.09% when using nitrogen-doped TiO_2 nanoparticles Ca-alginate-immobilized *P. chrysosporium*, while with the free and the immobilized *P. chrysosporium* they were 41.72% and 55.30% at pH 7.0, respectively (Hu et al, 2016). Saetang and Babel (2010b) also tested the pH effect (3-5) on the biodegradation of a medium-age leachate by immobilized fungi (*Trametes versicolor* or *Flavodon flavus*). The optimum pH was found to be 4 for both species. Very little change in pH was observed during the experiments, although no buffer was used. For example, colour removals at pH 4 for *T. versicolor* and *F. flavus* were around 35 and 30 %, respectively. These values decreased when pH values were increased to 5 (27 and 23%) or decreased to 3 (31 and 27%). Except for these two investigations, a fixed pH is employed and maintained constant during the biodegradation, with values ranging from 3.5 (Brito et al 2013,2012; Wichitsathian et al, 2004) to 6.2 (Kim et al, 2003). Faco and Santaella (2002) carried out the degradation of a mature leachate in a batch reactor without pH control (initial pH: 8.2) using *Aspergillus niger* and *Cladosporium herbarum*. The pH of the effluent was reported to be higher than that of the leachate, and the higher the residence time, the higher was this increase in the pH. This rise was of 6.2 – 12% for the inoculated reactor and of 5.3 – 13.5% for the

control, and was attributed to the degradation of volatile fatty acids in the leachate by the microorganisms and to the carbon dioxide desorption caused by the aeration. However, these authors reported a pH decrease using the same leachate and fungi, but with a continuous upflow reactor and adjusting the initial pH of the leachate to 5. In this case, this behaviour was attributed to the nitrification process and to the accumulation of orthophosphates in the reactor (Faco and Santaella, 2002).

Regarding the temperature, only Hu et al (2016) studied the effect of this parameter. They tested different temperatures during the degradation of a mature leachate by *Phanerochaete chrysosporium,* with the free fungus, Ca-alginate-immobilized and on Ca-alginate loaded with TiO_2. These authors reported that the removal efficiencies of TOC and NH_3–N increased sharply at temperatures ranging from 20 °C to 37 °C, and then declined slowly when the temperature increased further. In the case of the immobilized fungus, the increase in the removal efficiencies is not only due to improvement of its metabolic activity, but also to the swelling effect that occurred within the internal Ca- alginate structure at a higher temperature, which enabled organic pollutants to penetrate further into the structure (Hu et al, 2017).

In all other papers found in the bibliography, temperature was not a variable but was fixed and maintained approximately constant during all the experimentation. Typical temperatures selected range from room temperature (Bardi et al, 2017a,b; Brito et al 2012; Brito, 2013) to 37ºC (Hu et al, 2016,2017).

4.8. Other studies

Kalcikova et al. (2014) applied a crude enzyme filtrate from *Dichomitus squalens* to a medium-aged leachate and a mature one. They observed that the enzymatic treatment

was capable of purifying the medium-aged liquid, which had inhibited the growth and action of the Basidiomycete, but failed when used to treat the old leachate, which was, however, successfully purified by the fungus itself. Crude enzyme filtrates from *Ganoderma australe* and *Trichoderma harzianum* also proved their capacity to degrade the young leachates used by Wan Razarinah et al. (2014) and the theoretically mature one of Awasthi et al. (2017), respectively.

Finally, comparisons between fungal processes and bacterial techniques suggest that the former provide higher pollutant removal and lower membrane fouling than the latter (Amaral et al., 2017a; Gao et al., 2004; Ghosh et al., 2014; Reis et al., 2017; Wichitsathian et al., 2004), although this advantage is lost in certain cases, since the treated effluent is sometimes more toxic than the untreated one (Reis et al., 2017; Spina et al, 2018; Tigini et al., 2013).

Table 2. Purification of leachates by means of fungi.

Autor	Fungi	Measurements
Faco and Santaella (2002)	*Aspergillus niger* and *Cladosporium herbarum*	• COD removal and pH in batch reactors as a function of time. • Effect of glucose presence on the COD removal in continuous upflow reactors with adhered growth at several residence times. Nitrites, nitrates and orthophosphates when glucose was added.
Kim et al. (2003)	*Phanerochaete chrysosporium* IFO 31249	• pH, dissolved oxygen and removal of DOC, NH_4-N, color and soluble COD in three successive experiments: (i) adsorption on clinoptilolite followed by fungal degradation in an air-lift reactor, (ii) fungal degradation in the air-lift reactor alone and (iii) adsorption on clinoptilolite followed by fungal degradation in an air-lift reactor with continuous addition of 2 mL/min nitrogen limited medium to stimulate fungal growth.
Wichitsathian et al. (2004)	Bacterial and yeast sludges.	• COD and TKN removal in membrane bioreactors with bacteria or yeasts at two hydraulic retention times. • Molar mass distribution at the highest retention time • Membrane fouling
Gao et al. (2004)	*Phanerochaete chrysosporium*	• Effect of residence time, glucose concentration and NH_4-N content on the COD removal in a fixed bed bioreactor filled with fungi immobilized on cinder. • Comparison of reductions of COD, color and ammonia in the fixed bed fungal reactor whith those in a sequencing batch reactor filled with activated sludge.
Ellouze et al.(2008, 2009)	*Trametes trogii*, *Phanerochaete chrysosporium*, *Lentinus tigrinus* and *Aspergillus niger*.	• Time-dependence of COD removal and enzymatic activities for the four fungi and 50% leachate. At the end of the incubation, removals of toxicity, ammonia, phenols and hydrocarbons • Effect of leachate dilution on the COD removal by *A. niger* • Influence of NH_4-N on *T. trogii* and *P. chrysosporium* in media without leachate (Ellouze et al., 2009).

Saetang and Babel(2009)	*Trametes versicolor* BCC 8725 immobilized on polyurethane foam	• Effect of immobilization time, leachate dilution, presence of glucose and reaction time on biomass growth and on removal of COD, BOD_5 and color in a continuous reactor
Saetang and Babel (2010a)	*Trametes versicolor* BCC 8725 immobilized on polyurethane foam pieces	• Effect of glucose presence on color removal, biomass weigh and enzymatic activities during the reaction in a flask and on removal of both COD and BOD_5 at the end of it.
Saetang and Babel (2010b)	*Trametes versicolor* BCC 8725 and *Flavodus flavus* BCC 17421 immobilized on polyurethane foam pieces	• Effect of pH on decolorization • Effect of the C source, its concentration on decolorization, biomass growth and removal of COD and BOD_5
Saetang (2009)	Several leachates. *Trametes versicolor* BCC 8725 and *Flavodus flavus* BCC 17421 immobilized on polyurethane foam pieces	• Effect of pH, C source and its concentration on decolorization, biomass growth and removal of COD and BOD_5 • Reusability of the immobilized *T. versicolor*
Brito et al.(2012), Brito (2013)	*Saccharomyces cerevisiae*	• Effect of leachate dilution on cell concentration and COD removal for 48 and 96 h in flasks filled with two media (water or Sabouraud broth) • Cell concentration, suspended solids and rejections of COD, color and HS during the several acclimatization stages in a submerged membrane bioreactor. Cell

		concentration, suspended solids and removal of COD, NH_4-N, HS, Cl and P in the post-acclimatization. Membrane fouling.
Abdullah et al.(2013)	*Ganoderma australe*	• Effect of diluting the leachate on BOD_5, COD, NH_4-N and pH during ten cycles of operation in a glass column packed with 30 pieces of Ecomat-immobilized mycelia. • Enzymatic activities in a HA-free medium.
Tigini et al.(2013)	*Porostereum spadiceum* (MUT 1585)	• Time-evolution of decolorization and enzymatic activities • Ecotoxicity tests at the end
Tigini et al.(2014)	Three *Aspergillus*, one *Arthrinium*, two *Penicillium,* two *Pseudallescheria* and some basidiomycetes	• Effect of glucose on decolorizations given by the ten fungi
Gosh et al.(2014)	Fungus *Phanerochaete* sp ISTL01 and bacterium *Pseudomonas* sp. ISTDF1	• In flasks, effect of the type of C and N sources. Effect of concentration of the C and N sources and of reaction time on COD and color removals in 17 experiments carried out using Box–Behnken design and response surface methodology. • In a sequential bioreactor (fungal treatment first followed by bacterial treatment) at optimized conditions, overall COD and color removal.
Kalcikova et al.(2014)	Two leachates *Dichomitus squalens* MZKI B1233 Enzyme filtrate from this fungus	• Time-dependence of the toxicity, enzymatic activites, COD removal and DOC removal for the two leachates treated by the fungus. • Time-dependence of the toxicity, enzymatic activites, COD removal and DOC removal for the two leachates (diluted to 50%) treated by the enzyme filtrate.
Wan Razarinah et al.(2014)	*Ganoderma australe*, immobilized on Ecomat	• BOD_5, COD, NH_4-N and pH after a two-phase treatment, consisting of fungal degradation followed by the crude enzyme.

	Crude enzyme from this fungus	
Wan Razarinah et al.(2015)	*Trametes menziesii*, immobilized on Ecomat	• Evolution of BOD_5, COD, NH_4-N and pH with time
Hu et al.(2016)	*P. chrysosporium* BKMF-1767 (i) free, (ii) inmobilized on Ca-alginate or (iii) inmobilized together nitrogen-doped TiO_2 nanoparticles on Ca-alginate	• Effect of pH and temperature on the removal of TOC and of NH_4-N for free and immobilized fungi.
Hu et al.(2017)	*P. chrysosporium* BKM-F1767 (i) free, (ii) inmobilized on Ca-alginate or (iii) inmobilized together graphitic carbon nitride on Ca-alginate	• TOC removal for the free and immobilized fungi. Effect of light irradiation when the graphitic carbon nitride was present.
Bardi et al.(2017b)	*Bjerkandera adusta* MUT 2295, immobilized on polyurethane foam cubes	• pH, COD, BOD_5, color, MnP activity and biomass increase
Bardi et el.(2017a)	*Bjerkandera adusta* MUT 2295, free or immobilized on polyurethane foam cubes	• In flasks, with immobilized fungi: effect of the concentration of cellulose on MnP activity, COD removal and decolorization. • In flasks, with free fungi: effect of leachate dilution on MnP activity and COD removal • In packed bed bioreactors, with immobilized fungi: time evolution of COD removal when the co-substrate was glucose or cellulose.

Awasthi et al.(2017)	Enzyme filtrate from *Trichoderma harzianum* (FGCC#A29)	• Time evolution of COD and toxicity
Amaral et al.(2017b)	*Saccharomyces cerevisiae*	• COD, DOC, color, TN, NH_4-N and toxicity in the feed and in the permeate of a submerged membrane bioreactor during several acclimatization stages and the post-acclimatization phase. • Suspended solids, sludge particle size, soluble microbial products, extracellular polymeric substances and membrane fouling were also determined.
Amaral et al. (2017a)	Yeast sludge and bacterial sludge	• COD, color, NH_4-N and phosphorum in the feed and in the permeate of a submerged membrane bioreactor during the post-acclimatization stage. • Cell concentration in the acclimatization and the post-acclimatization phases. • Soluble microbial products, extracellular polymeric substances and membrane fouling during the post-acclimatization phase.
Reis et al.(2017)	Yeast sludge and bacterial sludge	• COD, color, TN, NH_4-N, chlorides, phosphorum and toxicity in the feed and in the permeate of a submerged membrane bioreactor during the post-acclimatization stage. • The same parameters after a nanofiltration of the permeate. • Chemicals present in the streams

5. CONCLUSIONS AND FUTURE RESEARCH

After reviewing the bibliography about fungal treatment of leachates and NOM-containing water, it can be concluded that fungi (and especially those of the white-rot type) turn out to be an attractive alternative for the treatment of these pollutants. These

microorganisms attack and decompose the compounds mainly by means of extracellular ligninolytic enzymes (Lac, MnP and LiP) and by adsorption, the first process being of greater interest because it avoids the need for further treatment of the biomass.

However, the effectiveness of fungal degradation depends on the complicated relationship between a long list of factors: the composition of the liquid waste, the presence of nutrient sources and their concentrations, pH, temperature, reaction time, the type of fungus, inoculation mode, and whether the microorganism is immobilized or not. Due to this, searching for the optimal conditions is a complex task, which should not be accomplished by optimizing "one-factor at a time", but by employing multivariate statistical techniques and experimental designs, such as the Box–Behnken design (Ghosh et al., 2014).

The high number of variables involved, as well as the high heterogeneity in terms of composition of both landfill leachates and NOM-containing waters, make it extremely difficult to compare different studies and obtain general conclusions. Due to this, a second reasonable suggestion for future work would be the definition of a "standard NOM-containing water" or, in particular, a "standard leachate" that could be generally accepted for experimental use by researchers in order to allow the direct and objective comparison of the removal efficiencies obtained in different studies. The availability of such a synthetic medium as a reference would also provide a more objective and quantitative scale of measurement for the "fungal treatability" of a specific real landfill leachate.

Another point to be borne in mind is that the procedure for fungal growth before its inoculation into the leachate is not normally optimized, but instead, investigators tend to simply follow that recommended by the supplier. In this regard, the acclimation strategies of the fungus to the environment of the leachate are not usually taken into

account, which probably causes a lag phase in the fungal growth after inoculation into the leachate. In time, the fungus adapts to the new medium, modifying its metabolism and increasing its assimilation rate. This means that reported removal rates obtained after only one cycle in batch experiments (a high percentage of the investigations here reported) are probably lower than those that would be observed during a hypothetical "real" situation (with a continuous process and the fungus completely acclimated to the leachate).

Another aspect that should receive more attention is the initial pH. As previously reported, this parameter is not optimized, but it is fixed at a certain value by the addition of acid to the always basic leachate. The initial determination of the optimum pH at which the fungal growth and or activity are maximum could involve not only a reduction in the hydraulic retention time, but also lower consumption of reagents, as well as a less acidic final effluent. Finally, certain aspects of the initial elimination of the leachate´s native microbiota (by heat or chloramphenicol addition, for example) require deeper discussion. On one hand, the previous sterilization of the leachate allows the exact determination of the true performance of the allochthonous fungal strains; on the other hand, the contribution of autochthonous fungi to global leachate removal is significant and should not be discounted, especially if an industrial application is the final aim (Faco and Santaella, 2002). Furthermore, the autochthonous leachate microbiota can be an excellent source for the screening and selection of new and more active fungal strains.

The mechanisms of the fungal degradation of both NOM-containing waters and leachates and the products formed during the process are areas where more knowledge is required, offering interesting lines of further research . The greatest effort in this direction was made by Reis et al (2017), who identified 145 organic pollutants (carboxylic acids, alcohols, aldehydes, amides, amines, ketones, esters, ethers, phenols, phosphates,

hydrocarbons, isocyanates, terpenes and halogenated compounds, among others) present in leachate, 27 of which were generated during the microbial degradation by *Saccharomyces cerevisiae*. The authors propose that these compounds play a key role in the final toxicity of the effluent. Hu et al (2017, 2016) reported that immobilized *Phanerochaete chrysosporium* presented an outstanding removal performance for almost all organic compounds in landfill leachate, especially for the volatile fatty acids and long-chain hydrocarbons. GC–MS analysis of metabolites detected at different stages of the treatment of a hypothetically medium-aged leachate by *Phanerochaete* sp. showed the degradation of products of lignin and polycyclic aromatic compounds (Gosh et al, 2014). Other authors have studied the evolution of the molecular weight distribution during fungal treatment, reporting a large increase in the proportion of the low molecular weight compounds and a preferential attack on the high molecular weight fraction, for both NOM and leachates (Lee, 2005; Wichitsathian et al 2004). Of equal importance, and related to the first suggestion for further investigation, is the development and use of kinetic models for fungus activity that are more complex that the mere calculation of average specific degradation rates, since such models are not available in the bibliography.

During the exploration of the reaction pathway and the kinetic model, it should be stressed that the abovementioned findings about leachate removals reported in the bibliography are the result of combining adsorption and biodegradation. One phenomenon has not been separated from the other because, in principle, both are of interest in a full-scale process (i.e., in a wastewater treatment plant, the activated sludge removes organic matter through adsorption and biodegradation). Thus, Hu et al. (2016) did not immobilize fungal biomass on Ca-alginate to improve biodegradation, but to increase biosorption. These authors even incorporated TiO_2 nanoparticles (Hu et al., 2016) and graphitic carbon nitride (Hu et al., 2017) in order to further adsorption and

photodegradation of the pollutants. However, the biodegradation as an independent process is a topic which should be considered, since it not only leads to higher removal of pollutants than adsorption, but to their destruction, thereby reducing the problems associated with the subsequent management of the excess fungal biomass.

Finally, further research is required, not only to better understand the fungal reactions or to optimize them, but also to evaluate the economic aspects of the various alternative scenarios and to compare them with each other and with non-fungal techniques employed nowadays.

6. ASSOCIATED CONTENT

The Supporting Information is available.

7. ACKNOWLEDGEMENTS

The authors are grateful for the financial support from the Employment, Industry and Tourism Office of Principality of Asturias (Spain) through project GRUPIN IDI/2018/000127. Authors also acknowledge the financial support from the Spanish Ministry of Economy and Competitiveness (MINECO) through Project CTM2015-63864-R and FEDER funds from European Union. We are very grateful to Prof. Dr. José Ramón Álvarez, from our department, for his help during the literature search.

8. REFERENCES

1 Abdullah, N., Wan Razarinah, W.A.R., Mahmood, N.Z., Taha, R.M. 2013. Treatment of
2 landfill leachate using *Ganoderma Australe* mycelia immobilized on Ecomat.
3 *Int. J. Environ. Sci. Dev.*, **4**(5), 483 - 487.
4 Al Mamun, A., Alam, M.Z., Mohd, N.A.N., Rashid, S.S. 2011. Adsorption of heavy
5 metal from landfill leachate by wasted biosolids. *Afr. J. Biotechnol.*, **10**(81),
6 18869 - 18881.
7 Amaral, M.C.S., Brito, G.C.B., Reis, B.G., Lange, L.C., Moravia, W.G. 2017a.
8 Comparison of commercial baker's yeast versus bacteria-based membrane
9 bioreactors for landfill leachate treatment. *Environ. Technol.*, 1-8.
10 Amaral, M.C.S., Gomes, R.F., Brasil, Y.L., Oliveira, S.M.A., Moravia, W.G. 2017b.
11 Performance evaluation of startup for a yeast membrane bioreactor (MBRy)
12 treating landfill leachate. *J. Environ. Sci. Health., Part A*, **52**(14), 1352-1360.
13 American Public Health Association (APHA)., Eaton, A. D., American Water Works
14 Association., & Water Environment Federation. (2005). Standard methods for
15 the examination of water and wastewater. Washington, D.C: APHA-AWWA-
16 WEF.
17 Anglada, Á., Urtiaga, A., Ortiz, I., Mantzavinos, D., Diamadopoulos, E. 2011.
18 Treatment of municipal landfill leachate by catalytic wet air oxidation:
19 assessment of the role of operating parameters by factorial design. *Waste*
20 *Manage.*, **31**(8), 1833-1840.
21 Awasthi, A.K., Pandey, A.K., Khan, J. 2017. Potential of fungus *Trichoderma*
22 *harzianum* for toxicity reduction in municipal solid waste leachate. *Int. J.*
23 *Environ. Sci. Technol.* , **14**, 2015 - 2022.
24 Bardi, A., Yuan, Q., Siracusa, G., Chicca, I., Islam, M., Spennati, F., Tigini, V., Di
25 Gregorio, S., Levin, D.B., Petroni, G., Munz, G. 2017a. Effect of cellulose as

co-substrate on old landfill leachate treatment using white-rot fungi. *Bioresour. Technol.*, **241**, 1067-1076.

Bardi, A., Yuan, Q., Tigini, V., Spina, F., Varese, G.C., Spennati, F., Becarelli, S., Di Gregorio, S., Petroni, G., Munz, G. 2017b. Recalcitrant compounds removal in raw leachate and synthetic effluents using the white-rot fungus *Bjerkandera adusta*. *Water* **9**.

Bhatnagar, A., Sillanpää, M. 2017. Removal of natural organic matter (NOM) and its constituents from water by adsorption – A review. *Chemosphere*, **166**, 497-510.

Bodzek, M., Surmacz-Gorska, J., Hung, Y.-T. 2006. Treatment of landfill leachate. 2 ed. in: *Handbook of Industrial and Hazardous Wastes Treatment*, (Eds.) L.K. Wang, Y.-T. Hung, H.H. Lo, C. Yapijakis, Marcel Dekker Inc. New York, pp. 1257-1320.

Brito, G.C.B. 2013. Evaluation of the performance of the use of membrane bioreactor inoculated with yeasts (*Saccharomyces cerevisiae*) in the treatment of sanitary landfill leachate. in: *Meio Ambiente e Recursos Hídricos*, Vol. Ph.D., Universidade federal de Minas Gerais. Belo Horizonte, pp. 185.

Brito, G.C.B., Amaral, M.C.S., Lange, L.C., Pereira, R.C.A., Santos, V.L., Machado, M. 2012. Treatment of landfill leachate in membranes bioreactor with yeast (*Saccharomyces cerevisiae*). *Procedia Eng.*, **44**, 934-938.

Collado, S., Laca, A., Días, M. 2013. Effect of intermediate compounds and products on wet oxidation and biodegradation rates of pharmaceutical compounds. *Chemosphere,* **92**, 2017-212**.**

Collado, S., Oulego, P., Súarez-Iglesias O. Díaz, M. 2018. Biodegradation of dissolved humic substances by fungi. *Appl. Microbiol. Biotechnol.*, **102** (8), 3497-3511.

1 Di Maria, F., Sisani, F. 2017 A life cycle assessment of conventional technologies for
2 landfill leachate treatment, Environ. Technol. Innovation, 8, 411-422
3 Ellouze, M., Aloui, F., Sayadi, S. 2008. Detoxification of Tunisian landfill leachates by
4 selected fungi. *J. Hazard. Mater.*, **150**(3), 642-648.
5 Ellouze, M., Aloui, F., Sayadi, S. 2009. Effect of high ammonia concentrations on
6 fungal treatment of Tunisian landfill leachates. *Desalination*, **246**(1), 468-477.
7 Facó, A.M., Santaella, S.T. 2002. Treatment of sanitary landfill leachate through a
8 biological process with fungi. *Proceedings of the XXVIII Interamerican*
9 *Congress of Sanitary and Environmental Engineering*, Cancún, Mexico.
10 Fernandes, A., Pacheco, M.J., Ciríaco, L., Lopes, A. 2015. Review on the
11 electrochemical processes for the treatment of sanitary landfill leachates: present
12 and future. *Appl. Catal., B*, **176–177**, 183-200.
13 Foo, K.Y., Hameed, B.H. 2009. An overview of landfill leachate treatment via activated
14 carbon adsorption process. *J. Hazard. Mater.*, **171**(1–3), 54-60.
15 Gao, H., Xu, H., Liu, Y. 2004. A study on treatment technology of landfill leachate by a
16 biofilm reactor with the white rot fungi. *Actae Scien. Circum.*, **24**(2), 309 - 314.
17 Gao, J., Oloibiri, V., Chys, M., Audenaert, W., Decostere, B., He, Y., Van Langenhove,
18 H., Demeestere, K., Van Hulle, S.W.H. 2015. The present status of landfill
19 leachate treatment and its development trend from a technological point of view.
20 *Rev. Environ. Sci. Bio/Technol.*, **14**(1), 93-122.
21 Garg, S.K., Modi, D.R. 1999. Decolorization of pulp-paper mill effluents by white-rot
22 fungi. *Crit. Rev. Biotechnol.*, **19**(2), 85-112.
23 Ghosh, P., Swati, Thakur, I.S. 2014. Enhanced removal of COD and color from landfill
24 leachate in a sequential bioreactor. *Bioresour. Technol.*, **170**, 10-19.

Ghosh, P., Thakur, I.S. 2017. Treatment of landfill leachate using fungi: an efficient and cost-effective strategy. in: *Developments in fungal biology and applied mycology*, (Eds.) T. Satyanarayana, S.K. Deshmukh, B.N. Johri, Springer. Singapore, pp. 341 - 357.

Gotvajn, A.Ž., Pavko, A. 2015. Perspectives on biological treatment of sanitary landfill leachate. in: *Wastewater Treatment Engineering*, (Ed.) M. Samer, InTech, pp. 115 - 151.

Grinhut, T., Hadar, Y., Chen, Y. 2007. Degradation and transformation of humic substances by saprotrophic fungi: processes and mechanisms. *Fungal Biol. Rev.*, **21**(4), 179-189.

Hu, L., Liu, Y., Zeng, G., Chen, G., Wan, J., Zeng, Y., Wang, L., Wu, H., Xu, P., Zhang, C., Cheng, M., Hu, T. 2017. Organic matters removal from landfill leachate by immobilized *Phanerochaete chrysosporium* loaded with graphitic carbon nitride under visible light irradiation. *Chemosphere*, **184**, 1071-1079.

Hu, L., Zeng, G., Chen, G., Dong, H., Liu, Y., Wan, J., Chen, A., Guo, Z., Yan, M., Wu, H., Yu, Z. 2016. Treatment of landfill leachate using immobilized *Phanerochaete chrysosporium* loaded with nitrogen-doped TiO_2 nanoparticles. *J. Hazard. Mater.*, **301**, 106-118.

Ikehata, K., Buchanan, I.D., Smith, D.W. 2004. Recent developments in the production of extracellular fungal peroxidases and laccases for waste treatment. *J. Environ. Eng. Sci.*, **3**(1), 1-19.

Kalčíková, G., Babič, J., Pavko, A., Gotvajn, A.Ž. 2014. Fungal and enzymatic treatment of mature municipal landfill leachate. *Waste Manage.*, **34**(4), 798-803.

Kim, Y.-K., Park, S.-K., Kim, S.-D. 2003. Treatment of landfill leachate by white rot fungus in combination with zeolite filters. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.*, **38**(4), 671-683.

Kjeldsen, P., Barlaz, M.A., Rooker, A.P., Baun, A., Ledin, A., Christensen, T.H. 2002. Present and long-term composition of msw landfill leachate: A review. *Crit. Rev. Environ. Sci. Technol.*, **32**(4), 297-336.

Kosobucki, P., Buszewski, B. 2014. Natural organic matter in ecosystems - A review Nova Biotechnologica et Chimica, **13**(2), 109-129

Kurniawan, T.A., Lo, W.-h., Chan, G.Y.S. 2006a. Physico-chemical treatments for removal of recalcitrant contaminants from landfill leachate. *J. Hazard. Mater.*, **129**(1–3), 80-100.

Kurniawan, T.A., Lo, W.-h., Chan, G.Y.S. 2006b. Radicals-catalyzed oxidation reactions for degradation of recalcitrant compounds from landfill leachate. *Chem. Eng. J.*, **125**(1), 35-57.

Kurniawan, T.A., Lo, W., Chan, G., Sillanpaa, M.E.T. 2010. Biological processes for treatment of landfill leachate. *J. Environ. Monit.*, **12**(11), 2032-2047.

Lee, M.K. 2005. Application of white-rot fungi for the biodegradation of natural organic matter in wastes. in: *School of Civil and Chemical Engineering*, Vol. Ph.D., RMIT University Melbourne. Melbourne, pp. 118.

Matilainen, A., Gjessing, E.T., Lahtinen,T., Hed, L., Bhatnagar, A., Sillanpää, M. 2011. An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment, *Chemosphere*, **83**(11), 1431-1442.

Oulego, P., Collado, S., Laca, A., Díaz, M. 2016. Impact of leachate composition on the advanced oxidation treatment. *Water Res.*, **88**, 389-402.

Pointing, S. 2001. Feasibility of bioremediation by white-rot fungi. *Appl. Microbiol. Biotechnol.*, **57**(1), 20-33.

Reemtsma, T. 2009. Determination of molecular formulas of natural organic matter molecules by (ultra-) high-resolution mass spectrometry: status and needs. *J. Chromatogr. A*, **1216**(18), 3687-3701.

Reis, B.G., Silveira, A.L., Tostes Teixeira, L.P., Okuma, A.A., Lange, L.C., Amaral, M.C.S. 2017. Organic compounds removal and toxicity reduction of landfill leachate by commercial bakers' yeast and conventional bacteria-based membrane bioreactor integrated with nanofiltration. *Waste Manage.*, **70**, 170-180.

Ren, Y., Yuan, Q. 2015. Fungi in landfill leachate treatment process. in: *Biodegradation and bioremediation of polluted systems - new advances and technologies*, (Eds.) R. Chamy, F. Rosenkranz, L. Soler, InTech, pp. 3 - 18.

Renou, S., Givaudan, J.G., Poulain, S., Dirassouyan, F., Moulin, P. 2008. Landfill leachate treatment: review and opportunity. *J. Hazard. Mater.*, **150**(3), 468-493.

Rojek, K., Roddick, F.A., Parkinson, A. 2004. Decolorization of natural organic matter by *Phanerochaete chrysosporium*: the effect of environmental conditions. *Water Sci. Technol.: Water Supply*, **4**(4), 175-182.

Saetang, J. 2009. Landfill leachate treatment by white rot fungi. in: *Sirindhorn International Institute of Technology*, Vol. Ph.D., Thammasat University, pp. 157.

Saetang, J., Babel, S. 2010a. Effect of glucose on enzyme activity and color removal by *Trametes versicolor* for high strength landfill leachate. *Water Sci. Technol.*, **62**(11), 2519-2526.

Saetang, J., Babel, S. 2009. Effect of leachate loading rate and incubation period on the treatment efficiency by *T. versicolor* immobilized on foam cubes. *Int. J. Environ. Sci. Technol.*, **6**(3), 457-466.

Saetang, J., Babel, S. 2010b. Fungi immobilization for landfill leachate treatment. *Water Sci. Technol.*, **62**(6), 1240-1247.

Sankaran, S., Khanal, S.K., Jasti, N., Jin, B., Pometto, A.L., Van Leeuwen, J.H. 2010. Use of filamentous fungi for wastewater treatment and production of high value fungal byproducts: a review. *Crit. Rev. Environ. Sci. Technol.*, **40**(5), 400-449.

Sen, S.K., Raut, S., Bandyopadhyay, P., Raut, S. 2016. Fungal decolouration and degradation of azo dyes: a review. *Fungal Biol. Rev.*, **30**(3), 112-133.

Sillanpää, M. (2015) *Natural Organic Matter in Waters: Characterization and Treatment Methods*. 1st Edition. Butterworth-Heinemann. Oxford

Singh, H. 2006. *Mycoremediation: fungal bioremediation*. John Wiley & Sons, Inc., Hoboken, New Jersey.

Solarska, S., May, T., Roddick, F.A., Lawrie, A.C. 2009. Isolation and screening of natural organic matter-degrading fungi. *Chemosphere*, **75**(6), 751-758.

Spina, F., Tigini, V., Romagnolo, A., Varese, G.C. 2018. Bioremediation of landfill leachate with fungi: autochthonous vs. allochthonous strains. *Life* 8(27), 1-15.

Thomson, J., Parkinson, A., Roddick, F.A. 2004. Depolymerization of chromophoric natural organic matter. *Environ. Sci. Technol.*, **38**(12), 3360-3369.

Tigini, V., Prigione, V., Varese, G.C. 2014. Mycological and ecotoxicological characterisation of landfill leachate before and after traditional treatments. *Sci. Total Environ.*, **487**, 335-341.

Tigini, V., Spina, F., Romagnolo, A., Prigione, V., Varese, G.C. 2013. Effective biological treatment of landfill leachates by means of selected white rot fungi. *Chem. Eng. Trans.*, **32**, 265 - 270.

Tortella, G.R., Diez, M.C., Durán, N. 2005. Fungal diversity and use in decomposition of environmental pollutants. *Crit. Rev. Microbiol.*, **31**(4), 197-212.

Vaverková, M.D., Adamcová, D., Radziemska, M., Voberková, S., Mazur, Z., Zloch, J. 2018. Assessment and evaluation of heavy metals removal from landfill leachate by *Pleurotus ostreatus*. *Waste Biomass Valorization*, **9**(3), 503 - 511.

Wan Razarinah, W.A.R., Zalina, M.N., Abdullah, N. 2014. Treatment of landfill leachate by immobilized *Ganoderma australe* and crude enzyme. *ScienceAsia*, **40**, 335 - 339.

Wan Razarinah, W.A.R., Zalina, M.N., Abdullah, N. 2015. Utilization of the white-rot fungus *Trametes menziesii* for landfill leachate treatment. *Sains Malays.*, **44**(3), 309 - 316.

Wichitsathian, B., Sindhuja, S., Visvanathan, C., Ahn, K.H. 2004. Landfill leachate treatment by yeast and bacteria-based membrane bioreactors. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.*, **39**(9), 2391-2404.

Yadav, M., Yadav, H.S. 2015. Applications of ligninolytic enzymes to pollutants, wastewater, dyes, soil, coal, paper and polymers. *Environ. Chem. Lett.*, **13**(3), 309-318.

Zhou, J.L., Banks, C.J. 1993. Mechanism of humic acid colour removal from natural waters by fungal biomass biosorption. *Chemosphere*, **27**(4), 607-620.

FUNGAL BIODEGRADATION OF LEACHATES AND NATURAL ORGANIC MATTER

Paula Oulego, Sergio Collado, Octavio Suárez-Iglesias, and Mario Díaz.

Table S1. Fungal decolorization of NOM

Table S2. Fungal decolorization of NOM according to the work of Lee (2005)

Table S3. Purification of leachates by means of fungi.

1

2 **References are the same than in the text**

3 **Table S1. Fungal decolorization of NOM**

Autor	Fungi, medium and experimental conditions	Comments and results
Thomson et al.(2004)	• Waksman's special medium without peptone, with NOM (30 mg C/L), inoculated with *Phanerochaete chrysosporium* ATCC 34541 and incubated at 37ºC in shake flasks at 120 rpm for up to 16 days.	• These experiments were part of a more general paper where the molecular mass distributions of NOM after depolymerization by several techniques were determined. • None is said on NOM adsorption by the fungal mycelium. The area of the size exclusion chromatography peaks decreased 1.3% at day 5, 13.5% at day 9 and 40% at day 16. After the incubation, it was seen that there was a preferential removal of the high-molecular weight compounds, although the modal mass did not change. New small peaks appeared in the low-molecular weight part of the chromatogram.
Rojek et al. (2004)	• The white-rot fungus *Phanerochaete chrysosporium* ATCC 34541 • Flasks containing 200 mL medium (modified Waksman or low nitrogen one), 10 – 480 mg/L NOM and pH fitted to 4.5. Inoculation with spore suspensions to give a final concentration of $2.2 – 2.5 \times 10^5$ spores/mL and incubation in an orbital shaker at 130 rpm at 36°C for 3–7 days. The modified Waksman medium consisted of 0.5 g/L $MgSO_4$, 1.0 g/L KH_2PO_4, 0 – 4 g/L glucose and 0–1 g/L NH_4Cl. The low nitrogen one was the same than that of Lee (2005).	• In a modified Waksman medium with 0.5 g/L NH_4Cl and 50 mg/L NOM incubated for 2 days, the initial glucose content was varied from 0 to 2 g/L. Decolorization was of 0% at 0 g/L, 38% at 0.8 g/L and 37% at 2 g/L. Glucose consumption was of 0% at 0 g/L, 99% at 0.8 g/L and 40% at 2 g/L • In a modified Waksman medium with 0.5 g/L NH_4Cl and 1 g/L glucose incubated for unspecified time, the initial NOM content was varied from 10 to 360 mg/L. Decolorization decreased almost linearly from 38% at 10 mg/L to 7% at 360 mg/L, whereas glucose consumptions were of 48% at 10 mg/L, 85% at 50 mg/L, 93% at 120 mg/L and 90% at 360 mg/L. Biomass growth followed the same trend that glucose consumption. • In a modified Waksman medium with 1.0 g/L NH_4Cl and 4 g/L glucose incubated for unspecified time, the initial NOM content was varied from 120 to 480 mg/L. Glucose consumption increased almost linearly from 38% to 90%, whereas decolorizations were of 36% at 120 mg/L, 42% at 240 mg/L and 40% at 360 mg/L. Biomass growth followed the same trend that glucose consumption.

		• In a modified Waksman medium under unspecified conditions, the NH_4Cl content was increased from 0 to 1 g/L. Decolorizations were of 18% at 0 g/L, 44% at 0.1 g/L, 50% at 0.6 g/L and 57% at 1 g/L. There was glucose consumption and biomass growth. Addition of 1.2 ppm Mn(II) did not increase the bleaching. • Incubation in nitrogen limited medium resulted in lower decolorization and greater fungal growth compared with Waksman medium. • Further tests were performed to determine the effect of pH and NaCl. When pH was increased from 3 to 5.8, the biomass growth and the glucose consumption increased, but the decolorization fell. When NaCl content rose from 0 to 80 g/L, decolorization was roughly constant up to 50 g/L and biomass weight slighlty decreased, making the NOM removed per unit of biomass to increase. However, beyond 50 g/L NaCl, there was a marked reduction of bleaching, fungal growth and NOM removed per unit of biomass, and at 80 g/L NaCl no decolorization was seen. • Further experiments on adsorption by live and inactivated pellets suggested that a 60 – 70% of the decolorization was due to biosorption.
Solarska et al. (2009)	• White-rot fungi *Trametes* sp, *Polyporus* sp., *Trametes versicolor* ATCC 7731 and *Bjerkandera adusta* • 500-mL Erlenmeyer flasks containing 200 mL of tap water with NOM (100 mg C/L) were inoculated with 10 g wet weight of pre-grown fungal pellets and incubated at 30ºC and 100 rpm for 230 h.	• For *T. versicolor*, decolorizations were of 17.5% at 15 h, 40% at 95 h and 25% at 230 h. pH took values of 7.5 at 0 h, 5 at 15 h, 5.4 at 95 h and 7.6 at 230 h. It excreted MnP, LiP and Lac, but the time-dependence of their activities was not reported. • For *Polyporus* sp., decolorizations were of 37% at 40 h, 40% at 85 h and 42% at 180 – 230 h, and pHs were of 7.5 at 0 h, 4.9 at 15 h, 4.7 at 40 h, 6.4 at 140 h, 5.8 at 170 h and 6.3 at 230 h. It excreted MnP and LiP, but the time-dependence of their activities was not given. • For *Trametes* sp., decolorizations of 26% at 40 h, 44% at 130 h and 35% at 230 h were achieved, whereas pH values of 7.6, 5.0, 5.15 and 7.0 were obtained at 0, 40, 120 and 230 h, respectively. Lac activity went through a maximum of 180 U/L at 120 h, whereas MnP activity reached its maximum at 75 h (24 U/L). No LiP was produced. • For *B. adusta*, pH values were of 6.8 at 0 h, 5.0 at 30 h, 6.35 at 70 h, 5.35 at 100 h and 7.5 at 220 h. Decolorizations and enzymatic activities are those of Figure 2a. • The maxima in bleaching were attributed to the release of secondary metabolites into the culture fluid, and the initial pH drop to generation of organic acids from the humic

matter present in the NOM. No explanation was given to the subsequent rise of pH with increasing time.
- Experiments with heat-killed mycelia probed that a 15-16% of the color removal in *Polyporus* sp. and *B. adusta* was due to adsorption, but only 4-6% in *Trametes* sp and *T. versicolor*. It is not clear if these numbers correspond to the máximum decolorizations or to 230 h.
- When compared with non-degraded samples, size exclusión chromatograms displayed a preferential reduction of the high-molecular-mass compounds, a decrease of the modal mass and formation of peaks at low molecular weights.

Table S2. Table S2. Fungal decolorization of NOM according to the work of Lee (2005)

Studied parameter	Fungi, medium and experimental conditions	Comments and results
Type of NOM	• Three types of NOM: the first one contained a 49% of very hydrophobic acids, the second one a 51% and the third one a 69%. • *Phanerochaete chrysosporium* ATCC 34541 • 200 mL of modified Waksman medium (2 g/L glucose, 0.5 g/L NH_4Cl, 1 g/L KH_2PO_4 and 0.5 $MgSO_4$) and 100 mg C/L NOM, inoculated with 10 mL of spore suspension (to attain a concentration of 1.0-1.5 x 10^5 spores/mL) and	• For the first NOM, the absorbance at 446 nm (color) decreased a 32%, the absorbance at 254 nm (conjugated and aromatic bonds) fell a 0.8%, the glucose consumption was of 0.75 g/L and the biomass generated of 58 mg. For the second NOM the absorbance at 446 nm decreased a 31%, the absorbance at 254 nm fell a 2.5%, the glucose consumption was of 0.75 g/L and the biomass generated of 57 mg. For the third NOM, the absorbance at 446 nm decreased a 38%, the absorbance at 254 nm fell a 9%, the glucose consumption was of 0.95 g/L and the biomass generated of 72 mg. • Size exclusion chromatography indicated a slight preferential attack to the high molecular weight fraction in the first and second NOM, and a considerable one in the third NOM. There was apparition of small new peaks in the low molecular weight range in the three cases.

	incubated at 36ºC and 130 rpm for 5 days.	• It was observed that the fungal pellets appeared brownish in color. This indicated that NOM molecules were bonded to the fungal mycelium and so adsorption of NOM to the biomass seemed to play a role in color removal.
Medium	• NOM containing a 69% of very hydrophobic acids • *P. chrysosporium* ATCC 34541 • 200 mL of medium and 100 mg C/L NOM, inoculated with 10 mL of spore suspension and incubated at 36ºC and 130 rpm for 14 days. The media were the modified Waksman one (as previously described, with a C:N ratio of 6), the Fahy one (25 g/L glucose, 0.5 g/L NH_4Cl, 2.5 g/L KH_2PO_4 and 1.25 $MgSO_4$, with a C:N ratio of 76) and a low nitrogen one (10 g/L glucose, 0.1 g/L NH_4NO_3, 1 g/L KH_2PO_4 and 0.5 $MgSO_4$, with a C:N ratio of 114)	• In the Waksman medium, decolorizations at 446 nm were of 36, 41 and 32% at 4, 10 and 14 days, respectively. Glucose consumptions were of 0.8, 0.9 and 1.4 g/L at 2, 4 and 14 days, respectively. The final dry weight of the generated biomass was of 100 mg. • In the Fahy medium, decolorizations at 446 nm were of 22, 32 and 27% at 4, 10 and 14 days, respectively. Glucose consumptions were of 1.8, 1.9 and 3.3 g/L at 2, 4 and 14 days, respectively. The final dry weight of the generated biomass was of 89 mg. • In the low nitrogen medium, decolorizations at 446 nm were of 21, 29 and 19% at 4, 10 and 14 days, respectively. Glucose consumptions were of 2.7, 3.3 and 4.1 g/L at 2, 4 and 14 days, respectively. The final dry weight of the generated biomass was of 80 mg. • The fungal pellets generated in the three culture media had a brown appearance indicating NOM adsorption to the cell wall. The decrease in decolorization beyond day 10 was attributed to desorption.
fungus	• NOM containing a 69% of very hydrophobic acids • *Phanerochaete chrysosporium* strains ATCC 34541 and ATCC 24725, *Trametes versicolor* strain ATCC 7731 and *Saccharomyces* species arbitrarily denoted 1, 2 and 3 (isolated from NOM concentrate) • 200 mL modified Waksman medium with 100 mg C/L NOM was	• For *P. chrysosporium* ATCC 34541, decolorizations at 446 nm were of 7, 37 and 38% at 1, 3 and 5 days, respectively. The absorbance fall at 254 nm after 5 days was of 9%. The overall glucose consumption was of 0.95 g/L and the final biomass weight of 72 mg. • For *P. chrysosporium* ATCC 24725, decolorizations at 446 nm were of 8, 34 and 36% after 1, 3 and 5 days, respectively. The absorbance fall at 254 nm after 5 days was of 9.6%. The overall glucose consumption was of 1.3 g/L and the final biomass weight of 72.5 mg. • For *T. versicolor* ATCC 7731, decolorizations at 446 nm were of 7, 58 and 59% after 1, 3 and 5 days, respectively. The absorbance fall at 254 nm after 5 days was of 17.3%. The overall glucose consumption was of 1.95 g/L and the final biomass weight of 138 mg.

	inoculated with either 10 mL spore suspension of white-rot fungi or a loopful of yeast from a malt-extract-agar culture. The mixtures were then incubated at 130 rpm and 36ºC for 5 days (white-rot fungi) or 30º C for 7 days (the yeast).	• For *Saccharomyces* sp. 1, decolorizations at 446 nm were of 4% at day 2, 10% at day 4 and 9.4% at day 7. The overall glucose consumption was of 0.8 g/L and the final biomass weight of 49.6 mg. • For *Saccharomyces* sp. 2, decolorizations at 446 nm were of 26% at day 2, 61% at day 4 and 61.6% at day 7. The overall glucose consumption was of 1.9 g/L and the final biomass weight of 220 mg. • For *Saccharomyces* sp. 3, decolorizations at 446 nm were of 6% at day 2, 22% at day 6 and 22.6% at day 7. The overall glucose consumption was of 1.7 g/L and the final biomass weight of 136 mg. • The color removal by the *Saccharomyces* species and *P. chrysosporium* was attributed predominantly to adsorption as indicated by the deep brown colorization of the biomass for them, whereas *T. versicolor* was light brown in color. Size-exclusion chromatograms showed that the *Saccharomyces* sp. 2 removed little NOM, preferentially of high molecular weight and did not generated peaks of low molecular weight. For the white-rot fungi, the chromatograms informed of a considerable reduction of NOM (mainly in the high molecular weight range as well) and formation of organic fragments of low molecular weight, being both effects very noticiable for *T. versicolor*. • At day 3, LiP, MnP and Lac activities were measured for the three white-rot fungi. LiP was the main enzyme secreted by *P. chrysosporium*, where strains ATCC 34541 and ATCC 24725 produced 2.4 U/L and 1.5 U/L LiP, respectively. Very-low activities of MnP and Lac were found. For *T. versicolor*, Lac was the main enzyme (3.36 U/L), whereas the activities of LiP and MnP were around 0.23 U/L.
Temperature, inoculation mode and glucose concentration	• NOM containing a 69% of very hydrophobic acids • *Trametes versicolor* ATCC 7731 • 200 mL modified Waksman medium with 100 mg C/L NOM inoculated with fungal spore suspensions and incubated at 30ºC or 36ºC and 130 rpm for 9 days. Further experiments were carried out at 30ºC in a high-	• For 30ºC, a modified Waksman medium and inoculation with spores, decolorizations at 446 nm were of 6.5% at day 3, 23% at day 5 and 73% at day 9; absorbance decreases at 254 nm were of 6.3% at day 4, 19.7% at day 6 and 55.2% at day 9; glucose consumptions were of 0.36 g/L at day 1, 0.67 g/L at day 5 and 1.92 g/L at day 9; pH values were of 5.4, 4.8, 2.9 and 3.2 at days 0, 3, 5 and 9, respectively. The final biomass weight was of 245 g. Activities of LiP, MnP and Lac at an unspecified day were of 1.2, 6.0 and 17 U/L, respectively. • For 36ºC, a modified Waksman medium and inoculation with spores, decolorizations at 446 nm were of 58% at day 3 and of 51% at day 9; absorbance decreases at 254 nm were of

glucose modified Waksman medium (5 g/L glucose instead of 2) inoculated with spores for 9 days and at 30ºC in a modified Waksman medium inoculated with three malt-extract agar plugs (each 1 cm^2) instead of with spores, for 8 days.

17% at day 3 and of 20.5% at day 9; glucose consumptions increased from 0 g/L at day 0 to 1.95 g/L at day 3 and then, remained unchanged; pH values were around 5.5 from 0 to 1 day, fell to 3.2 at day 2 and then remained roughly constant. LiP, MnP and Lac activities at an unspecified day were of 0.2, 0.2 and 3 U/L, respectively. The color removal at this temperature was partially due to adsorption as indicated by the brown coloration of the biomass compared to the cultures at 30ºC, where the pellets were cream in color.

- For 30ºC, a glucose-rich modified Waksman medium and inoculation with spores, decolorizations at 446 nm were of 13.7, 40.2 and 49% after 3, 4 and 9 days, respectively. Absorbance reductions at 254 nm were of 2.8% at day 2, 21% at day 4 and 21.2% at day 9. Glucose consumptions were of 1.2, 2.3 and 2.7 g/L at 3,5 and 9 days, respectively. pH values were close to those obtained with normal Waksman medium inoculated with spores at the same temperature, and the final biomass weight was of 377 mg. There was brown colorization of the mycelium. Enzymatic activities took values of 0.5 U/L LiP, 1.9 U/L MnP and 1.2 U/L Lac at an unspecified day.
- For 30ºC, a modified Waksman medium and inoculation with plugs, decolorizations at 446 nm were of 14.5, 35 and 52% after 4, 5 and 8 days, respectively. Absorbance reductions at 254 nm were of 1.9% at day 2, 4.7% at day 4 at day 5 and 29% at day 8. Glucose consumptions were of 0.0, 0.77, 0.82 and 1.23 g/L at 2, 4, 5 and 8 days, respectively, and the null comsumption in the first two days was attributed to the presence of readily assimilable C in the malt-extract-agar plugs. pH values took values of 5.3 at day 0, 5.1 at day 2, 3.7 at day 5 and 3.4 at day 8. Lac activity was higher than that obtained at 30ºC in the modified Waksman medium inoculated with spores. The biomass formed and that on the plugs was creamy brown, indicating that adsorption was not very important.
- Size-exclusion chromatography was only carried out in the modified Waksman medium inoculated with spores. Chromatograms displayed higher removals at 30ºC than at 36ºC, a preferential attack to the high-molecular-weight fractions, no change of modal masses and apparition of new peaks at low molecular masses. These new peaks were more abundant at 36ºC than at 30ºC, probably because the fungus was able of removing them at low temperatures.

NOM concentration	• NOM containing a 69% of very hydrophobic acids • *Trametes versicolor* ATCC 7731 • 200 mL modified Waksman medium with 100 – 700 mg C/L NOM, inoculated with three malt-extract agar plugs (each 1 cm^2) and incubated at 30ºC and 130 rpm for 10 days.	• Decolorizations at 446 nm were of 71.5% at 100 mg/L C, 62.4% at 300 mg/L C, 51.5% at 600 mg/L C and 18.9% at 700 mg/L C. Reductions of absorbance at 254 nm were of 46.2% at 100 mg/L C, 22.4% at 300 mg/L C, 25.7% at 600 mg/L C and 4.5% at 700 mg/L C. Glucose consumptions were of 1.56 g/L at 100 mg/L C and 1.82 g/L at 700 mg/L C, going through a maximum of 1.92 g/L at 300 mg/L C. • LiP activities were of 9.4 and 5.6 U/L at 100 and 300 mg/L C, being zero at 400 mg/L C and beyond. MnP activities monotonically decreased from 17.8 U/L at 100 mg/L C to 12.5 U/L at 700 mg/L C. Lac activites were 34.4 U/L at 100 mg/L C, 90 U/L at 700 mg/L C and went through a maximum of 195 U/L at 600 mg/L C.
Presence of Tween 20	• NOM containing a 69% of very hydrophobic acids • *Trametes versicolor* ATCC 7731 • 200 mL high-glucose modified Waksman medium with 500 mg/L NOM, inoculated with three malt-extract agar plugs (each 1 cm^2) and agitated continuously at 30ºC and 130 rpm for 13 days. In some cultures, 0.5% Tween 80 was added.	• In preliminary tests without NOM, Tween 20 increased the activity of the ligninolytic enzymes. However, in presence of NOM, results were different. • Without Tween 80, the decolorization at 446 nm, the glucose consumption and the Lac activity were respectively 48%, 2.2 g/L, and 109 U/L after 5 days, 54%, 2.8 g/L and 187 U/L after 9 days, 82%, 4.5 g/L and 330 U/L after 11 days and 86%, 4.6 g/L and 347 U/L after 13 days. The fall of absorbance at 254 nm after the same days was of 11, 19, 42 and 43%. • When Tween 80 was added, the decolorization at 446 nm, the glucose consumption and the Lac activity were respectively 47.7%, 3.1 g/L, and 75 U/L after 5 days, 57%, 3.3 g/L and 146 U/L after 9 days, 81.5%, 4.8 g/L and 235 U/L after 11 days and 81.6%, 4.9 g/L and 286 U/L after 13 days. The fall of absorbance at 254 nm after the same days was of 10, 19, 39 and 39%.
Presence of wheat bran	• NOM containing a 69% of very hydrophobic acids • *Trametes versicolor* ATCC 7731 • 200 mL high-glucose modified Waksman medium supplemented with 4.5 g/L wheat bran and 0.5% (v/v) Tween 80, and NOM (final concentration of 100, 600 and 700 mg	• Tween 20 and wheat bran were added because in preliminary tests without NOM, they increased the activity of the ligninolytic enzymes. • Decolorization at 446 nm was of 79, 61 and 17% at 100, 600 and 700 mg C/L, respectively. Reduction of absorbance at 254 nm was of 55% at 100 mg/L, 31% at 600 mg/L and 4% at 700 mg/L. Glucose consumptions of 5.28 g/L at 100 mg/L, 5.11 g/L at 600 mg/L and 5.20 g/L at 700 mg/L were obtained. • When eliminating the NOM removal due to adsorption by the wheat bran and the colorization due to the bran itself, absorbance decreases at 446 nm became 61, 50 and 12%

	C/L) were prepared and each was inoculated with three fungal plugs. The cultures were incubated at 30ºC and 130 rpm for 13 days	for 100, 600 and 700 mg/L C, which were lower than those obtained in the modified Waksman medium (2 g/L glucose) without Tween 20 and without wheat bran. • At 100 mg C/L, LiP, MnP and Lac activities were of 14.8, 19.8 and 13.5 U/L, respectively. At 600 mg C/L, LiP, MnP and Lac activities were of 34.6, 25.6 and 95.6 U/L, respectively. At 700 mg C/L, LiP, MnP and Lac activities were of 5.4, 16.5 and 29.6 U/L, respectively.
Degradation ability of the enzymes	• NOM containing a 69% of very hydrophobic acids • Filtered extracellular culture fluid, enriched in Lac. • The reaction mixture contained 4.0 mL of the culture fluid, 3.0 mL of Na_2HPO_4-citric acid buffer and 3 mL of a NOM solution (final concentration of 300 mg C/L). pH was varied between 3 and 7 at 30ºC, and temperature was varied from 30 to 80ºC at pH 4.5. The incubation time was not specified.	• The filtered extracellular culture fluid was obtained from a modified Waksman medium with 4.5 g/L wheat bran, 0.5% Tween 80, *T. versicolor* plugs and no NOM at 9 days. • For 30ºC, decolorization at 446 nm was of 17% at pH 3, 5% at pH 7 and went through a maximum of 22.3% at pH 4.5. Reduction of absorbance at 254 nm followed a similar pattern: 2.8% at pH 3, 5.6% at pH 4.5 and -5.6% at pH 7. • For pH 4.5, reduction of absorbance at 446 nm was of 22.4% at 30ºC and 40ºC, 25.2% at 50ºC and 10.3% at 60ºC. At 70 and 80ºC, the absorbance was not modified. Reduction of absorbances at 254 nm went through a maximum of 6.3% at 60ºC, being 5.8% at 30ºC and -3.3% at 80ºC.

Table S3. Purification of leachates by means of fungi.

Autor	Fungi, medium and experimental conditions	Comments and results
Faco and Santaella (2002)	• Leachate from the West Metropolitan landfill of Caucaia (Fortaleza, Brazil), which worked since 1993. COD of 433 – 4700 mg/L and pH of 8.25 – 8.45. • *Aspergillus niger* and *Cladosporium herbarum* • Two batch reactors (one inoculated and one of control) with residence times of 2, 4, 8 and 16 days, placed in an aseptic chamber to minimize bacterial contamination. The culture media contained 5.2 g/L glucose, 0.4 g/L KH_2PO_4, 0.4 g/L K_2HPO_4, 0.4 g/L $MgSO_4$, 0.3 g/L NaCl and 0.05 g/L $CaCl_2$. The fungal reactors were also treated with the antibiotic chloramphenicol	• In the inoculated reactor, COD removals of 40, 48, 57 and 62% were achieved for 2, 4, 8 and 16 days, respectively. In the control one, removals were of 51% for 2 days, 27% for 4 days, 76% for 8 days and 86% for 16 days. The better performance of the control reactor was attributed to the presence of microorganisms in the leachate, already adapted, that degradated the organic matter. • The pH of the effluent was higher than that of the leachate, and the higher the residence time, the higher the increase. This rise was of 6.2 – 12% for the inoculated reactor and of 5.3 – 13.5% for the control one, and was attributed to the degradation of volatile fatty acids of the leachate by the microorganisms.
Faco and Santaella (2002)	• Leachate from the West Metropolitan landfill of Caucaia (Fortaleza, Brazil) with 433 – 4700 mg/L COD, 1500 – 2200 mg/L nitrate, 0.2 - 3.7 mg/L nitrite and 0.7 – 2.3 mg/L orthophosphate. • *Aspergillus niger* and *Cladosporium herbarum* • Continuous upflow reactor with adhered growth, with and without 0.5 g/L glucose. Unspecified dimensions and residence times of 2, 4 and 6 days.The pH of the effluent was fixed to around 5.	• Without glucose, COD removals in the fungal reactor were of 34, 65 and 29% for 2, 4 and 6 days, respectively, whereas they were of 13, 39 and -1% for the same days in the control reactor. With glucose, COD removals in the fungal reactor were of 66, 84 and 63% for 2, 4 and 6 days, respectively, whereas they were of 56, 72 and 47% for the same days in the control reactor. The maxima were attributed to the adaptation of the fungi to the hydraulic conditions or to the replacement of the *A.niger* and *C. herbarum* by other species from the leachate (*Rhizoupus* sp. and *Staphylococcus aureus*).

		• In the fungal reactor with glucose there was production of nitrates for 2 and 6 days (day 4 was not analyzed), production of nitrites for 2 days but consumption for 4 days (day 6 was not analyzed), consumption of ortophosphates for 2 and 6 days, but production for 4 days. pH decreased in all the cases. These changes were attributed to the nitrification process and to accumulation of ortophosphates in the reactor.
Kim et al.(2003)	• Leachate from a landfill in Chunchon (Korea), started in 1991 and closed in 1995, with 785 – 985 mg/L COD (730 – 960 mg/L soluble), 55 – 120 mg/L BOD_5 (48 – 115 mg/L soluble), 290 – 407 mg/L DOC and 1041 – 1605 mg/L NH_4-N • *Phanerochaete chrysosporium* IFO 31249 • An adsorption column (5.1 cm diameter, 50 cm height, filled with 0.5 L of Clinoptilolite) and an air-lift fungal reactor of 5.2 L effective volume (85 cm high, 44 cm diameter with an inner draft tube of 3.4 cm diameter and 70 cm height, concentrically placed). Three runs were carried out: adsorption followed by fungal reactor with residence time of 30 h, only fungal reactor with residence time of 30 h and adsorption followed by fungal reactor with continuous addition of 2 mL/min nitrogen limited medium to stimulate fungal growth (residence time of 20 h because the medium increases the flow rate). Airflow around 190 – 220 mL/min	• The overall experiment lasted for 45 days. Adsorption plus fungal degradation lasted from day 6 to day 18. Fungal degradation alone was performed from day 18 to day 21. Adsorption plus fungal degradation plus nitrogen limited medium was implemented from days 31 to 45. • The pH in the effluent of the fungal reactor during all the experiment was of 5.6 – 6.2 (almost the same than the influent). The average dry weight of the biomass and the concentration of dissolved oxygen were 1579 mg/L biomass and 4.0 mg/L O_2 for days 6 – 18, 1380 mg/L biomass and 4.4 mg/L O_2 for days 18 – 31 and 1800 mg/L biomass and 2.9 mg/L O_2 for days 31 – 45. • Average removals were 53% soluble COD, 44% DOC, 81% NH_4-N and 51% color for days 6 – 18, 21.5% soluble COD, 21.5% DOC, 16.8% NH_4-N and 31.4% color for days 18 – 21 and 65% soluble COD, 62% DOC, 81% NH_4-N and 59% color for days 31 – 45. Adsorption removed most of the ammoniacal nitrogen (75% in days 6 – 18 and 70% in days 31 – 45) and a considerable part of color (32% in days 6 – 18 and 16% in days 31 – 45) • The adsorption step increases the soluble COD to DOC ratio and the soluble BOD_5 to soluble COD ratio in the final effluent.
Wichitsathian et al.(2004)	• A mixed stream from two sites: Pathumthani Sanitary landfill and Ram-Indra Transfer Station, Thailand. It contained 8000 mg/L COD, 1900 mg/L TKN, 1700	• In the bacterial reactor working without ammonia stripping, COD removals were of 62% for 16 h and of 60% for 24 h, whereas TKN removals took values of 25% for both residence times. When ammonia

	mg/L NH_4-N, 3200 mg/L BOD_5 and pH 8. As the ratio of TKN to NH_4-N was so close to the unity, the ammonium content was assumed to be equal to the total Kjeldahl nitrogen. • Bacterial and yeast sludges. • The experiments were conducted in two membrane bioreactors operated in parallel, one with the bacterial culture and the other with the yeast culture (5 L, polyethylene microfiltration hollow fiber membranes with a pore size of 0.1 μm and surface area of 0.42m^2, continuously aerated with to maintain 3–5 mg/L dissolved oxygen and periodically backwashed with air). The pH was maintained at 3.5–3.8 for the yeast system and 6.8–7.0 for the bacterial device. Suspended solid concentrations were around 10000 mg/L. Initially, the bioreactors were operated without ammonia stripping and later, with it. Hidraulic retention times of 16 and 24 h were considered.	stripping was applied, COD removals increased to 72% for 16 h and to 76% for 24 h, whereas TKN removals reached a 81% for 16 h and a 86% at 24 h (35% and 42%, respectively, if only the removal in the bioreactor is considered). • In the yeast reactor working without ammonia stripping, COD removals were of 67% for 16 h and of 63% for 24 h, whereas TKN removals took values of 27 and 28% for 16 and 24 h, respectively. When ammonia stripping was applied, COD removals increased to 72% for 16 h and to 76% for 24 h, whereas TKN removals reached a 84% for 16 h and a 86% at 24 h (30% and 43%, respectively, if only the removal in the bioreactor is considered). • For both the bacterial and yeast bioreactors (retention time of 24 h and ammonia stripping) the molecular weight of COD and BOD fractions was determined by membrane filtration. In both reactors, the biodegradation highly increased the proportion of the the low molecular weight compounds (5 kDa<) and reduced the proportion of high molecular weight substances (>50 kDa) • Membrane fouling was more frequent in the bacterial bioreactor than in the yeast one. The first membrane cleaning was done after 63 days of operation in the bacterial device, while it was done after 80 days in the yeast set-up. The transmembrane pressure in the bacterial apparatus increased significantly compared to that of the yeast system.
Gao et al.(2004)	• Leachate from the Shangai Laogang landfill, with 2087 mg/L COD, 106 mg/L BOD_5, 974 mg/L NH_4-N and pH 8.5 • *Phanerochaete chrysosporium* • Fixed bed bioreactor (1005 mL with an effective volume of 845 mL) filled with fungi immobilized on cinder (granules of 1 cm^3). Aereation, with an air/water ratio of 20:1. Temperature of 30ºC. A solution	• Due to the previsible negative impact of the high ammoniacal nitrogen, three ways of removing it were assayed: air stripping with pH 11 and a ratio air/water of 3000, air stripping with pH 12 and a ratio air/water of 4000 and precipitation as magnesium ammonium phosphate (ratio Mg/N/P of 1/1/1), being the last one the most effective (final concentration of 84 mg/L NH_4-N) • Three doses of polyferric coagulant (600, 800 and 1000 mg/L) were also tested for decreasing the COD, being 800 mg/L the selected one. The

	containing glucose was added to the leachate stream to improve the fungal nutrition.	combination of the magnesium ammonium phosphate with the polyferric coagulation led to a COD of 800 mg/L. • When the residence time was increased from 66 to 97 h (at a constant concentration of 500 mg/L glucose), the COD removal rose from 25 to 37%, but a subsequent increase to 128 h did not improve the last figure. • When the glucose concentration was increased from 500 to 2000 mg/L (at a constant residence time of 97 h), COD removal rose from 37 to 47.6%, but a subsequent increase to 5000 mg/L glucose did not improve the last removal. • Finally, the pretreatment effectiveness was decreased to observe the influence of increasing amounts of NH_4-N and COD in the COD removal. Glucose concentration and residence time were set to 2000 mg/L and 97 h, respectively. As expected, the effect was negative. This removal decreased from 47.6% at 800 mg/L COD and 84 mg/L NH_4-N to 32% at 925 mg/L COD and 500 mg/L NH_4-N, reaching a 24.2% at 1025 mg/L NH_4-N and 1050 mg/L COD.
Gao et al.(2004)	• Pretreated leachate from the Shangai Laogang landfill, with around 806 mg/L COD and 86.5 mg/L NH_4-N. • Comparison of activated sludge in a sequencing batch reactor (12 h residence time, unspecified dimensions) with the previously described fungal fixed bed reactor (97 h, 2000 mg/L glucose)	• In the activated sludge process, there was a COD fall of 16.7%, an ammonia reduction of 22.1%, a decolorization of 11.1% and a HS decrease of 13.5%. • In the fungal reactor, there was a COD fall of 46.8%, an ammonia reduction of 68.5%, a decolorization of 43.2% and a HS decrease of 27.8%
Ellouze et al.(2008, 2009)	• Landfill leachate from Djebel Chekir (south-west of Tunis,Tunisia), with 15000- 20000 mg/L COD, 728 mg/L NH_4-N, 2.84 g/L phenols, 3.1 g/L hydrocarbons and BOD_5/COD around 0.51. • White-rot fungi *Trametes trogii*, *Phanerochaete chrysosporium*, *Lentinus tigrinus* and *Aspergillus niger*. • For *P. chrysosporium* and *L. tigrinus*, 1 L flasks with 100 mL static cultures (2 g/L KH_2PO_4, 0.14 g/L $CaCl_2$, 0.7 g/L $MgSO_4 \cdot 7H_2O$, 0.07 g/L $FeSO_4 \cdot 7H_2O$, 0.0462	• Most of the experiments were done with leachate diluted to 50%. *L. tigrinus* gave COD removals of 67.5% at day 3 and of 90.6% at day 15. *P. chrysosporium* gave COD removals of 49.8% at day 7 and of 68.8% at day 15, *T. trogii* of 40.4% at day 3 and 79.8% at day 15, and *A. niger* of 63.3 and 81.6% at days 7 and 15, respectively. • Enzyme activities went through maxima with time. For *L. tigrinus*, 380 U/L Lac at day 4 and 35 U/L MnP at day 9; for *T. trogii*, 200 U/L Lac at days 7-9, 50 U/L MnP at day 9 and 48 U/L LiP at day 9 as well; for *P. chrysosporium*, 115 U/L MnP at day 9 and 77 U/L LiP at day 11.

	g/L $ZnSO_4 \cdot 7H_2O$, 0.035 g/L $MnSO_4 \cdot 7H_2O$, 0.007 g/L $CuSO_4 \cdot 5H_2O$, 1 g/L yeast extract, 0.0025 g/L thiamine, 0.4 mmol/L veratryl alcohol, 10 g/L glycerol as carbon source and 20 mmol/L diammonium tartrate as nitrogen source), buffered to pH 5.5 with disodium tartrate, inoculated with 2 mL of mycelial suspension, incubated for 15 days and flushed with pure oxygen. • For the other two fungi, 1 L Erlenmeyer flasks with 100 mL medium buffered to pH 5.5, shaken at 150 rpm and 30ºC for 15 days. Mycelia of *T. trogii* were inoculated in a medium with 10 g/L glucose, 9 g/L soya peptone, 2 g/L diammonium tartrate, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L KCl, 1 mL/L trace elements solution, 0.3 mmol/L CuSO4 and 3% ethanol. Spores of *A. niger* were inoculated in a medium with 0.5 g/L $(NH_4)_2SO_4$ and 2 g/L KH_2PO_4	• At the end of the treatment, *A. niger* gave removals of toxicity, ammonia, phenols and hydrocarbons of 60, 79, 100 and 35%. The oter three fungi gave removals of 81% for toxicity, 91-94% for ammonia, 100% for phenols and higher than 99% for hydrocarbons. • *A. niger* was selected for experiments with 10, 30, 50, 70 and 100% leachate. Removal of COD were of 31 – 57% for day 3 and of 71 – 90% after 19 days. There was no clear concentration dependence for a given reaction time. • Further experiments were done on the influence of NH_4-N on *T. trogii* and *P. chrysosporium* in media without leachate (Ellouze et al., 2009). It was found that a concentration of NH_4Cl reaching 2 g/L did not affect fungal growth nor enzyme secretion, but 5 g/L of ammonium chloride caused inhibitor effect for both fungal growth and enzyme production.
Saetang (2009) Saetang and Babel (2009)	• Leachate from a pipe of the Nonthaburi landfill site (Thailand), with 2989 ADMI color units, 96512 mg/L COD, 48900 mg/L BOD_5, 20 mg/L NH_4-N and pH 5.7. • *Trametes versicolor* BCC 8725 immobilized on polyurethane foam for 4 or 15 days. • 4 cycles in a continuous reactor (each cycle lasted for 5 days).This apparatus was constructed with acrylic sheets with the inner dimension of 5 cm width, 28.7 cm length and 35 cm height for a total liquid volume of 3 L. Three baffles were placed as partitions in each reactor to have an up-down flow as shown in order to increase the contact time. Air diffusers were put at the bottom of each compartment. The leachate flow was adjusted to 0.4 mL/min. This flow was also selected for the internal recycle of a part of the treated leachate. pH was fitted to	• Pollution removal and biomass weight increased with the number of cycles, but tended to a plateau. • For 4-day immobilized fungi, raw leachate and without glucose, there were 11% color removal, 12% BOD_5 removal, 14% COD removal and 28.6 mg biomass after cycle 1 and 31% color removal, 25% BOD_5 removal, 23% COD removal and 42.3 mg biomass after cycle 4. When 3 g/L glucose was added to the medium, the results were 29% color removal, 24% BOD_5 removal, 21% COD removal and 51.2 mg biomass after cycle 1 and 67% color removal, 52% BOD_5 removal, 42% COD removal and 65.8 mg biomass after cycle 4. • For 4-day immobilized fungi, 5-times diluted leachate and without glucose, there were 16% color removal, 15% BOD_5 removal, 13% COD removal and 32.0 mg biomass after cycle 1 and 39% color removal, 35% BOD_5 removal, 25% COD removal and 52.6 mg biomass after cycle 4. When 600 mg/L glucose was added to the medium, the results

	4 and sometimes, glucose was added. There were 200 pieces of foam per compartment (total 800 pieces)	were 39% color removal, 36% BOD_5 removal, 35% COD removal and 60.0 mg biomass after cycle 1 and 78% color removal, 60% BOD_5 removal, 54% COD removal and 80.9 mg biomass after cycle 4. • For 15-day immobilized fungi, raw leachate and without glucose, there were 14% color removal, 15% BOD_5 removal, 16% COD removal and 63.9 mg biomass after cycle 1 and 32% color removal, 30% BOD_5 removal, 25% COD removal and 69.2 mg biomass after cycle 4. When 3 g/L glucose was added to the medium, the results were 32% color removal, 24% BOD_5 removal, 23% COD removal and 69.8 mg biomass after cycle 1 and 69% color removal, 54% BOD_5 removal, 46% COD removal and 78.5 mg biomass after cycle 4. • For 15-day immobilized fungi, 5-times diluted leachate and without glucose, there were 15% color removal, 14% BOD_5 removal, 13% COD removal and 61.2 mg biomass after cycle 1 and 40% color removal, 37% BOD_5 removal, 27% COD removal and 71.9 mg biomass after cycle 4. When 600 mg/L glucose was added to the medium, the results are those of Figure 2b.
Saetang and Babel (2010a), Saetang (2009)	• Leachate from a pipe of the Nonthaburi landfill site (Thailand), and storaged for one year at 4ºC. It contained 2074 ADMI color units, 69580 mg/L COD, 38100 mg/L BOD_5, 1568 mg/L NH_4-N and pH 7.9. • White-rot fungus *Trametes versicolor* BCC 8725 immobilized on polyurethane foam pieces (1×1×1 cm) for 4 days • 250-mL Erlenmeyer flasks with 100 mL leachate, 10 pieces of polyurethane foam, 0 or 3 g/L glucose and pH fixed to 4. Incubation under sterile conditions at ambient air temperature (30–33ºC) for 12 days.	• Color removal and biomass weigh increased with incubation time, but tended to a plateau. • Without glucose, there were 3.41% color removal and 2.2 mg biomass after 2 days, 11.62% color removal and 40.5 mg after 6 days, 12.19% color removal and 46.1 mg after 9 days and 12.21% color removal and 47.3 mg after 12 days. With glucose, the numbers were 6.53% color removal and 7.6 mg biomass after 2 days, 50.51% color removal and 114.8 mg after 6 days, 57.93% color removal and 194.2 mg after 9 days and 58.55% color removal and 209 mg after 12 days. • LiP, MnP and Lac were produced, and enzyme activities went through maxima with time at 6 h. Without glucose, these maxima were 102 U/L LiP, 437 U/L MnP and 781 U/L Lac. With glucose, they were 384 U/L LiP, 1241 U/L MnP and 2534 U/L Lac. After 12 h, activities fell to 55

		U/L LiP, 320 U/L MnP and 536 U/L Lac without glucose and to 175 U/L LiP, 458 U/L MnP and 1140 U/L Lac with glucose. • Without glucose, BOD_5 removal and COD removal after 12 days were of 12.8% and of 10.5%, respectively. With glucose, there was 37% BOD_5 removal and 40% COD removal.
Saetang and Babel (2010b), Saetang (2009)	• Leachate from a pipe of the Nonthaburi landfill site (Thailand), with 900 ADMI color units, 34560 mg/L COD, 5600 mg/L BOD_5, 182.10 mg/L TKN and pH 5.33. • White-rot fungi *Trametes versicolor* BCC 8725 and *Flavodus flavus* BCC 17421 immobilized on polyurethane foam pieces for 4 days • 100 mL leachate with 10 pieces of foam, pH 3 – 5 and 0 - 3 g/L glucose, cassava or corn starch, incubated at 150 rpm and ambient air temperature (30 – 33ºC) for 5 - 16 days.	• At an unspecified pH (4 or 5.33) biomass growth of the two fungi was measured. For a given C source, the higher the concentration, the higher the growth. After 16 days, the biomass of *T. versicolor* was 237.8 mg with 3 g/L glucose, 226.7 mg with corn starch and 134.8 mg with cassava. The biomass of *F. flavus* was 222.3 mg with 3 g/L glucose, 207.5 mg with corn starch and 159.8 mg with cassava. • At a constant pH and without C source, color removal increased with incubation time, but tended to a plateau. After 5 days, these removals for *T. versicolor* were 31.78% at pH 3, 33.45% at pH 4 and 28.66% at pH 5. For *F. flavus*, removals were 27.75% at pH 3, 29.64% at pH 4 and 24.96% at pH 5. • At pH 4 and external C sources, decolorizations were measured. They increased with time, reaching a plateau beyond days 7 – 9. The higher the concentration of C source, the higher the decolorization. After 15 days, *T. versicolor* caused 78.1% decolorization for 3 g/L glucose, 73.96% for 3 g/L cassava and 66.47% for 3 g/L corn starch. *F. flavus* caused a 73.05% decolorization for glucose, 68% for cassava and 60.08% for corn starch. BOD_5 and COD removals were also measured at the end of the experiments with 3 g/L C source. For *T. versicolor* they were 68.84% BOD_5 removal and 57.2% COD removal with glucose, 54.96% BOD_5 removal and 54.61% COD removal with corn starch, 50.12% BOD_5 removal and 52.73% COD removal with cassava and 35.25% BOD_5 removal and 48% COD removal without external C source. For *F. flavus* they were 65.6% BOD_5 removal and 52.19% COD removal with glucose, 51.63% BOD_5 removal and 49.92% COD removal with corn starch, 47.82% BOD_5 removal and 48.76% COD

		removal with cassava and 33.6% BOD_5 removal and 43% COD removal without external C source. • Further experiments with *T. versicolor* with 4 and 5 g/L glucose at pH 4 did not improve considerably the removal of color, BOD_5 and COD achieved with 3 g/L (Saetang, 2009).
Saetang (2009)	• Leachate from a large stabilization pond of the Nonthaburi landfill site (Thailand), with 600 ADMI color units, 4870 mg/L COD, 2100 mg/L BOD_5, 1542 mg/L TKN and pH 8.31. • White-rot fungi *Trametes versicolor* BCC 8725 and *Flavodus flavus* BCC 17421 immobilized on polyurethane foam pieces for 4 days • 100 mL leachate with 10 pieces of foam, pH 3 – 7 and 0 - 3 g/L glucose, cassava or corn starch, incubated at 150 rpm and ambient air temperature (30 – 33ºC) for 5 - 15 days.	• pH, C-source and time dependences of decolorization were similar to those of Saetang and Babel (2010b). • After 5 days, the maximum decolorization was achieved at pH 5 for *T. versicolor* (31.04%) and at pH 4 for *F. flavus* (29.86%) without external C source. • After 15 days at the optimum pH, *T. versicolor* gave 64.91% color removal, 43% BOD_5 removal and 57.2% COD removal for 3 g/L glucose, 56.98% color removal, 40% BOD_5 removal and 54.6% COD removal for 3 g/L cassava and 60.93% color removal, 41% BOD_5 removal and 55.3% COD removal for 3 g/L corn starch. *F. flavus* gave 60.96% color removal, 39.2% BOD_5 removal and 55.5% COD removal for 3 g/L glucose, 53.6% color removal, 27.6% BOD_5 removal and 50.8% COD removal for 3 g/L cassava and 57.32% color removal, 35% BOD_5 removal and 53.6% COD removal for 3 g/L corn starch.
Saetang (2009)	• Leachate from a garbage truck in Nonthaburi (Thailand), with 600 ADMI color units, 1728 mg/L COD, 625 mg/L BOD_5, 216 mg/L TKN and pH 3.85. • White-rot fungi *Trametes versicolor* BCC 8725 and *Flavodus flavus* BCC 17421 immobilized on polyurethane foam pieces for 4 days	• pH, C-source and time dependences of decolorization were similar to those of Saetang and Babel (2010b). pH 4 and 3 g/L glucose were the optimum conditions for treating the leachate. *T. versicolor* performed better than *F. flavus*. • The experiment with *T. versicolor*, pH 4 and 3 g/L g glucose was repeated for three cycles of seven days each, in order to test the reusability of the immobilized fungi. In the first cycle, decolorization

	• 100 mL leachate with 10 pieces of foam, pH 3 – 5 and 0 - 3 g/L glucose, cassava or corn starch, incubated at 150 rpm and ambient air temperature (30 – 33ºC) for 5 - 15 days.	increased from 13.03% at day 1 to 65.36% at day 5 and then to 68.5% at day 7. In the second cycle, it rose from 12.41% at day 1 to 63.92% at day 5 and to 66% at day 7. In the third cycle, the values were 10.45, 47.38 and 49.98% at days 1, 5 and 7, respectively. When corn starch was added instead of glucose, decolorizations were 1 – 4% lower (4 – 7% in the third cycle). When cassava was utilized, they were 3 – 8% lower (9 – 15% in the third cycle). If no additional C source was added, color removals were 3 – 36% lower than those with glucose.
Brito (2013)	• Landfill leachate from Macaúbas (Sabará, Brazil), working from 2007. Air stripping was performed in order to decrease the high amounst of NH_4-N • *Saccharomyces cerevisiae* • Erlenmeyer flasks with 1 g lyophilized yeast bread and 100 mL of leachate diluted to 0, 20, 40, 60 and 100% in water or in Sabouraud broth (10 g/L special peptone and 20 g/L dextrose). The initial concentration of yeast was around 1×10^7 CFU/mL. Incubation in sterile conditions at 150 rpm and 30ºC for 96 h.	• After 48 h in water, there were 2.41×10^7 CFU/mL at 0% leachate, 2.23×10^7 CFU/mL at 20% leachate and 5.96×10^3 CFU/mL at 100%. COD removals were of 4.2% at 20% leachate, -136% at 60% leachate and -89% at 100% leachate. Cell concentrations below 1×10^7 CFU/mL were attributed to fungal death and negative COD removals to the lysis of the dead cells and release of their organic compounds in the media. • After 96 h in water, there were 2.6×10^7 CFU/mL at 0% leachate, 1.14×10^8 CFU/mL at 20% leachate and 7.92×10^2 CFU/mL at 100%. COD removals were of 62% at 20% leachate, -10% at 80% leachate and -3% at 100% leachate. The better performance with regards to that after 48 h was attributed to a better acclimatization of the fungi. • After 48 h in broth, there were 2.5×10^7 CFU/mL at 0% leachate, 2.6×10^5 CFU/mL at 80% leachate and 1.6×10^6 CFU/mL at 100%. COD removals were of 44% at 20% leachate, -22% at 80% leachate and -5% at 100% leachate. The better performance with regards to water after 48 h was due to the nutrients of the broth. • After 96 h in broth, there was a decrease from 5.7×10^8 CFU/mL at 0% leachate to 1.75×10^6 CFU/mL at 100% leachate. COD removals also fell: 97% at 20% leachate, 46% at 60% and 80% leachate and -14% at 100% leachate. • Despite of the sterilized conditions, other microorganisms were detected, probably because they were present in the leachate

Brito et al.(2012), Brito (2013)	• Landfill leachate from Macaúbas (Sabará, Brazil), working from 2007 with variable concentration. Air stripping was performed in order to decrease its high amounts of NH_4-N. • The yeast *Saccharomyces cerevisiae* • Submerged membrane bioreactor, consisting of hollow fiber membranes of polyetherimide, with 0.04 m^2 of area and 0.5 μm of pore size (useful volume of 9.60 L). Experiments were carried out in two phases: acclimatization and post-acclimatization. In the first one, the feed was leachate diluted to 20% in water with 3 g/L of Sabouraud broth. The leachate concentration was progressively incremented to 40, 60, 80 and 100% with 3 g/L broth (around 20 days per concentration). Then, the concentration of broth decreased to 2, 1 and 0 g/L. At day 150, it started the post-acclimatization (from day 150 to 380). Operational conditions were: infinite sludge age, constant permeate flux of 5 L/m^2.h, air flow rate of 12.5 Nm/h, pH 3.5, temperature of 25 – 30ºC and hydraulic retention time of 48 h. Backwash was performed every 15 min with duration of 15 s and chemical cleaning when the transmembrane pressure was around 0.5 bar.	• In the acclimatization step, there was an intense contamination by wild filamentous fungi and non filamentous colonies, which also contributed to the biodegradation. In this phase, for 20% leachate and 3 g/L broth, the feed contained around 1235 color units, 4312 mg/L COD and 566 mg/L HS, being their average rejections of 70, 68 and 47%, respectively. For 40% leachate and 3 g/L broth, the feed contained 1443 color units, 4334 mg/L COD and 739 mg/L HS being their rejections of 77, 74 and 60%, respectively. For 60% leachate and 3 g/L broth, the feed contained 2100 color units, 4185 mg/L COD and 772 mg/L HS being their rejections of 84, 75 and 78%, respectively. For 80% leachate and 3 g/L broth, the feed contained 4433 color units, 6425 mg/L COD and 798 mg/L HS being their rejections of 91, 78 and 77%, respectively. For 100% leachate and 3 g/L broth, the feed contained 4759 color units, 6296 mg/L COD and 895 mg/L HS being their rejections of 91, 79 and 81%, respectively. For 100% leachate and 2 g/L broth, the feed contained 3729 color units, 3666 mg/L COD and 561 mg/L HS being their rejections of 89, 76 and 69%, respectively. For 100% leachate and 1 g/L broth, the feed contained 3300 color units, 3792 mg/L COD and 453 mg/L HS being their rejections of 86, 68 and 71%, respectively. • Average suspended solids in the acclimatization stage were 10000 mg/L for 20% leachate (concentrations of microorganisms not determined), 3500 mg/L for 40% leachate (9×10^9 CFU/mL *S. cerevisiae*, 4×10^8 CFU/mL filamentous fungi and 9×10^6 CFU/mL non filamentous colonies), 3000 mg/L for 60% leachate (3×10^{10} CFU/mL *S. cerevisiae*, 6×10^9 CFU/mL filamentous fungi and 8×10^7 CFU/mL non filamentous colonies), 4500 mg/L for 80% leachate (6×10^{10} CFU/mL *S. cerevisiae*, 3×10^9 CFU/mL filamentous fungi and 2×10^7 CFU/mL non filamentous colonies), 5000 mg/L for 100% leachate (8×10^{11} CFU/mL *S. cerevisiae*, 3×10^9 CFU/mL filamentous fungi and 9×10^8 CFU/mL non filamentous colonies), 4500 mg/L for 2 g/L broth (5×10^{12} CFU/mL *S. cerevisiae*, 8×10^{11} CFU/mL filamentous fungi and 3×10^{11} CFU/mL non filamentous colonies) and 5000 mg/L for 1 g/L broth (2×10^{12} CFU/mL *S.*

		cerevisiae, 1×10^{11} CFU/mL filamentous fungi and 8×10^{8} CFU/mL non filamentous colonies). • In the post-acclimatization, for a leachate with 1546 – 5891 color units, 1552 – 6899 mg/L COD, 206 – 1575 mg/L HS, 375 – 1562 mg/L NH_4-N, 1799 – 4898 mg/L chlorides and 19 – 58 mg/L phosphorum, removals of 54 – 95% color, 47 – 96% COD, 51 – 97% HS, 23 -88% ammonia, 25 – 90% Cl and -6 – 92% P were obtained. Suspended solids increased from 2000 mg/L at day 150 to 11000 at day 320, and then decreased to 6500 mg/L at day 380. Concentrations of *S. cerevisiae* (9.16×10^{10} – 4.48×10^{12} CFU/mL) and non filamentous fungi (2.25×10^{9} – 8.64×10^{11} CFU/mL) tended to increase with time, whereas the amount of filamentous colonies seemed to oscilate around a constant value (2×10^{11} CFU/mL) • It was reported lower extracellular polymeric substances production when compared with literature data for conventional membrane bioreactors, and lower fouling. The average permeability of the system was 37 L/m^2·h
Abdullah et al. (2013)	• Leachate from a pond at a sanitary landfill, with 3500 – 3470 mg/L BOD_5, 3580 – 3240 mg/L COD, 30.90 – 22.20 mg/L NH_4-N and pH 8.05. • The white-rot fungus *Ganoderma australe* • Reaction in a glass column (40 mm diameter and 500 mm height) packed with 30 pieces of Ecomat-immobilized mycelia. Immobilization was carried out in 250-mL Erlenmeyer flasks containing 50 mL of sterile glucose-yeast-malt-peptone medium, four pieces of Ecomat (2 x 2 cm squares, 1 mm thickness) and 5 mL of mycelium suspension, agitated at 100 rpm on an orbital shaker for 4 days.	• Ten cycles of operation at room temperature. Each cycle consisted of passing 1 L of raw or diluted (50%) leachate through the column at a flow rate of 20 mL/min. • BOD_5 was not efficiently removed in any cycle. COD removals were of 1.83 – 22.79% in raw leachate and of 16.77 – 51.62% in diluted one. The highest removal of ammoniacal nitrogen was achieved at the eighth cycle (45.95% for the diluted leachate and 30.90% for the raw one). pH slightly increased after each cycle. • Enzymal activities were measured by four days, but in a medium free of leachate. MnP was the most active, followed by Lac.

Tigini et al. (2013)	• Effluent sampled after the biological oxidation treatment of landfill leachates. It was dark colored, with 2532 mg/L COD, 408 mg/L NH_4-N and pH of 8.5. The effluent was taken to a more acidic value (around 5) and a low glucose amount (0.1 g/L) was added, because the original stream did not provide environmental conditions for fungal growth. • *Porostereum spadiceum* (MUT 1585) • Twenty portions (5 mm diameter) taken from the margins of a fungal colony in agarised malt extract medium were used as inoculum in 500-mL flasks containing 200 mL of a high nitrogen content medium (10 g/L glucose and 3.8 g/L yeast extract). After 7 days, the culture broth was replaced with 100 mL of effluent and the cultures were incubated for 7 days, at 25 °C and 120 rpm.	• Decolorizations at 1, 24, 96 and 168 h were 12, 40, 56 and 52%, respectively. The initial increase was attributed to adsorption. Peroxidase activities were of 0, 140 and 70 U/L at 0, 24 and 168 days, whereas Lac activities were almost negiligible. • Ecotoxicity tests of the leachates with *Pseudokirchneriella subcapitata* and *Lepidium sativum* showed that the fungal treatment increased the toxicity of the degradated leachate when compared to that of the raw leachate at pH 5. When the comparison was performed with the raw leachate at pH 8.5, then toxicity for *P. subcapiata* increased and toxicity for *L. sativum* decreased.
Tigini et al. (2014)	• Leachate from the central part of Italy, with 6166 mg/L COD, 4209 mg/L BOD_5 and pH 8.10. • Three *Aspergillus* (*A. fumigatus* MUT 4050, *A. tubingensis* MUT 1288 and *A. sydowii* MUT 1290), one *Arthrinium* (*A. sphaerospermum* MUT 777), two *Penicillium* (*P. brevicompactum* MUT 793, *P. corylophilum* MUT 784), two *Pseudallescheria* (*P. boydii* MUT 1269, MUT 721) and some basidiomycetes (*B. adusta* MUT 765, *P. sanguinea* MUT 1284, *Flammulina velutipes* MUT 1275) • Multiwell containing 2.5 mL wastewater, with or without 1 g/L glucose as C source, inoculated with mycelium discs (3 mm diameter) and incubated at 25ºC and 150 rpm for 20 days in the dark.	• Without glucose, decolorizations (as decrease of absorbance in the visible range) varied between 26 and 7%, and followed the decreasing order MUT 777 > MUT 1275 > MUT 1284 > MUT 721 > MUT 765 > MUT 4050 > MUT 1269 > MUT 784 > MUT 1290 > MUT 793 • With glucose, decolorizations varied between 34% and 7%, and followed the decreasing order MUT 1284 >MUT 721 >MUT 1269 > MUT 784 > MUT 1288 ≥ MUT 4050 > MUT 765 > MUT 1290 > MUT 773 > MUT 777. • Within the experimental error, only *P. sanguinea* MUT 1284 and *P. brevicompactum* MUT 793 showed a significant improvement of the decolorization rate in the presence of glucose.

Gosh et al. (2014)	• Leachate from Okhla landfill (South Delhi, India) with 8120 coloring units, pH 8.3 and 29020 mg/L COD • Fungus *Phanerochaete* sp ISTL01 and bacterium *Pseudomonas* sp. ISTDF1 • Erlenmeyer flasks with 7.8 g/L $Na_2HPO_4 \cdot 2H_2O$, 6.8 g/L; KH_2PO_4, 0.2 g/L $MgSO_4$, 0.085 g/L $NaNO_3$, 0.05 g/L $Ca(NO_3)_2 \cdot 4H_2O$, 0.5 – 1.5 g/L C source, 0.1 – 0.5 g/L N source, 20% v/v landfill leachate and pH 4 (fungi) or pH 8 (bacteria), inoculated with suspensions containing 2×10^5 spores/mL (fungi) or 5×10^4 CFU/mL (bacteria) and incubated at 30ºC on a rotary shaker (rpm 150) for 48 – 240 h days	• 17 experiments were carried out using Box–Behnken design and response surface methodology for three variables (C source, N source and duration). The response variables were COD and color removals. • The tested C sources were sucrose, dextrose, sodium citrate and sodium acetate, whereas the N sources were tryptone, yeast extract, sodium nitrate and ammonium nitrate. Dextrose was found to be the best C source for both strains, whereas tryptone was the most suitable N source for *Pseudomonas* sp. and yeast extract for *Phanerochaete* sp. • Optimized conditions for *Pseudomonas* sp., were 1.5 g/L dextrose, 0.387 g/L tryptone and 184.33 h (the model predicted 45.22% COD and 27.52% color removal), whereas for *Phanerochaete* sp. they were 1.13 g/L dextrose, 0.21 g/L yeast extract and 184.38 h (predicting removals of 55.76% COD and of 31.59% color) • Further experiments in a sequential bioreactor (fungal treatment first followed by bacterial treatment) of 15 L (effective volume of 10 L) at optimized conditions led to 76.9% COD reduction and 45.4% color removal.

Kalcikova et al. (2014)	• Two leachates: the first one from a landfill which was approximately 30 years old and was still active with continuous waste disposal (3263 mg/L COD, 1075 mg/L DOC, 1090 mg/L BOD_5 and 519 mg/L NH_4-N), and the second one, from a landfill operated between 1960 and 2001, but that was used since its closure for disposal of municipal waste and co-disposal of different wastes from tannery industry (1086 mg/L COD, 467 mg/L DOC, 27 mg/L BOD_5 and 732 mg/L NH_4-N). • White-rot fungus *Dichomitus squalens* MZKI B1233 • The treatments were prepared by inoculating 100 mL of nitrogen-limited medium with 50% v/v of landfill leachate without N and C source, but with beech wood sawdust, in 250 mL Erlenmeyer flasks with 5% v/v of mycelial suspension, and by incubating them on a rotary shaker (150 rpm) under 28ºC for 7 days.	• The first leachate did not support the growth of fungus at all. The COD and DOC did not decrease less than 2% in 4 days of the experiment and no Lac and MnP were produced. • For the second leachate, there were 835 mg/L DOC and 1740 mg/L COD at day 0, 448 mg/L DOC and 748 mg/L COD at day 2, 377 mg/L DOC and 661 mg/L COD at day 4 and 327 mg/L DOC and 696 mg/L COD at day 7. Enzymatic activities were 0 U/L of both Lac and MnP at day 0, 1.2 U/L Lac and 2.1 U/L MnP at day 2, 2.8 U/L Lac and 2.6 U/L MnP at day 4 and 1.2 U/L Lac and 2.4 U/L MnP at day 7. Inhibitions of *Sinapis alba* were 91.9, 80.4, 90.5 and 90% at days 0, 2, 4 and 7 respectively, wheras the inhibitions of *Aliivibrio fischeri* were 68.3, 44.8, 34.9 and 38.3% after 0, 2, 4 and 7 days, respectively.
Kalcikova et al. (2014)	• The two previously mentioned leachates • Crude enzyme filtrate, mainly containing Lac and MnP of the white-rot *Dichomitus squalens* • Mixture of crude enzyme filtrate and 50% v/v leachate, incubated on a rotatry shaker at 150 rpm, 28ºC and in the dark for 5-7 days.	• The concentration of leachate in the mixture (50%) was determined after previous inhibition tests of Lac, with concentrations ranging from 10 to 90% v/v. • For the first leachate, DOC, COD, inhibition of *Sinapis alba* and inhibition of *Aliivibrio fischeri* decreased in 5 days from 588 mg/L, 1635 mg/L, 82.5% and 84.3%, respectively, to 297 mg/L, 791 mg/L, 48.2% and 0% . The Lac activity fell from 738 to 351 U/L (although it seems that it passed through a tiny maximum at day 2) and MnP activities were of 152, 176 and 83 U/L at days 0, 1 and 5, respectively. • For the second leachate, only 23% of organic matter (COD and DOC) was removed after 7 days. However, it was comparable to the decrease of organic content in the blank control and thus it was assessed as not biodegradable by ligninolytic enzymes.

Wan Razarinah et al. (2014)	• Leachate from a sanitary landfill, with 4166 mg/L BOD_5, 5980 mg/L COD, 30.9 mg/L NH_4-N and pH 8.14 • *Ganoderma australe*, immobilized on Ecomat during 4 days in a glucose-yeast-malt-peptone medium, and crude enzyme from this white-rot fungus. • Experiments consisted of two phases. In the first phase, in 250-mL Erlenmeyer culture flasks, 125 mL of leachate was treated with immobilized *G. australe* on Ecomat. The flasks were then agitated on an orbital shaker for seven days at 28°C at 150 rpm. In the second phase, the treated leachate from the first phase was collected and subsequently treated with 10 U/mL of crude enzyme at 4 h of exposure on an orbital shaker at 80 rpm.	• After treatment with immobilized *G. australe*, the biodegraded leachate contained 2070 mg/L BOD_5, 4080 mg/L COD, 43.2 mg/L NH_4-N and pH 9.11. • When this effluent was treated with crude enzyme, 1730 mg/L BOD_5, 2570 mg/L COD, 11.7 mg/L NH_4-N and pH 8.87 were obtained. • The full decreases after the two-phase treatment were of 58% BOD_5, 57% COD and 62% ammoniacal nitrogen.
Wan Razarinah et al. (2015)	• Leachate from an active sanitary landfill in Malaysia, with 11265 – 11530 mg/L BOD_5, 13240 – 14740 mg/L COD, 15.45 – 20.4 mg/L NH4-N and pH 7.86 – 7.99. • White-rot fungus *Trametes menziesii*, immobilized on Ecomat during 4 days in a glucose-yeast-malt-peptone medium • 250 mL Erlenmeyer culture flasks with 125 mL of leachate and immobilized *T. menziesii,* agitated on an orbital shaker for 7 - 28 days at 28ºC at 150 rpm.	• BOD_5 removal increased monotonically with time, from 82% at day 7 to 93.48% at day 28. COD removal was maximum of 24.66% at day 7 and then decreasing, being 0.91% after 14 days and negative at day 28 (-7.12%). There was no reduction but production of ammoniacal nitrogen (the concentration of the degraded effluent after 28 days was of 39.9 mg/L NH_4-N). The value of pH showed that the longer the incubation time, the medium would become more alkaline.
Hu et al. (2016)	• Leachate from a landfill area of Heimifeng in Changsha (China), with 6140 mg/L COD, 558 mg/L BOD_5, 1890 mg/L TOC and 1856 mg/L NH_4-N	• The effect of COD concentration (100 – 400 mg/L) and biosorbent amount (10 - 40 g/L) was only studied with fungi inmobilized together nitrogen-doped TiO_2 nanoparticles on Ca-alginate, where not only bisorption and biodegradation took place, but also adsorption on titania

	• White-rot basidiomycete *P. chrysosporium* BKMF-1767 (i) free, (ii) inmobilized on Ca-alginate or (iii) inmobilized together nitrogen-doped TiO_2 nanoparticles on Ca-alginate. • 250 mL Erlenmeyer flasks containing 100 mL water and leachate, 100 – 400 mg/L COD, pH 3 – 8 and 10 – 40 g/L biosorbent, incubated at 20 – 45ºC in orbital shakers (150 rpm) for 72 – 96 h.	and photodegradation. However, the extent of each phenomenon was not separated. • For pH 3 – 8, 200 mg/L COD, 20 mg/L biosorbents and 37ºC and an unspecified time, TOC removal went through maxima at pH 6 and NH_4-N removal peaked at pH 7. These maxima were 40.15% TOC and 41.72% NH_4-N for free fungi, 54.97% TOC and 55.3% NH_4-N for fungi inmobilized on Ca-alginate and 73.49% TOC and 72.09% NH_4-N for fungi inmobilized together nitrogen-doped TiO_2 nanoparticles on Ca-alginate • For 20 - 45ºC, 200 mg/L COD, pH 6 and 20 g/L of biosorbent and an unspecified time, TOC and NH_4-N removals went through maxima at 37ºC. The maximum removal was achieved by fungi inmobilized together nitrogen-doped TiO_2 nanoparticles on Ca-alginate (65.45%, TOC reduction and 61.51% NH_4–N decrease), whereas free fungi gave the worst results.
Hu et al. (2017)	• Leachate from the refuse landfill of Heimifeng in Changsha (China), with 6250 mg/L COD, 630 mg/L BOD_5, 1920 mg/L TOC and 2045 mg/L NH_4-N • White-rot basidiomycete *P. chrysosporium* BKM-F1767 (i) free, (ii) inmobilized on Ca-alginate or (iii) inmobilized together the photocatalyst graphitic carbon nitride on Ca-alginate. • 250 mL Erlenmeyer flasks containing 100 mL leachate with 100 mg/L TOC, incubated at pH 6, 150 rpm and 37ºC for 96 h.	• For an individual graphitic carbon nitride weight of 0.10 g and an individual *P. chrysosporium* weight of 0.90 g, TOC removals under lighting were of 74.99% for the immobilized fungus with the photocatalyst, 44.83% for the immobilized fungus alone, 39.98% for the free fungus, 21.44% for the immobilized photocatalyst and 24.95% for the free photocatalyst. TOC removals without lighting were of 32.18% for the immobilized fungus with the photocatalyst, 42.01% for the immobilized fungus alone, 40.77% for the free fungus, 5.74% for the immobilized photocatalyst and 5.36% for the free photocatalyst. • The effect of TOC content (50 – 200 mg/L), reusability (3 cycles) and individual weight of fungus (0.5 – 2.5 g) or of photocatalyst (0.06 – 0.14

		g) were only studied with fungi inmobilized together the photocatalyst on Ca-alginate, where not only bisorption and biodegradation took place, but also photodegradation. However, the extent of each phenomenon was not separated.
Bardi et al. (2017b)	• Leachate from Brady Road landfill (Winnipeg, Canada), with 1636 mg/L COD, 704 mg/L NH_4-N, 150 mg/L BOD_5 and pH 7.6 • *Bjerkandera adusta* MUT 2295, immobilized on polyurethane foam cubes of 2 cm^3 for 7 days in flasks containing glucose and yeast extract broth. • 500 mL flasks containing 160 mL effluent and 2 cubes at 150 rpm, 10 days and room temperature. Glucose (1 g/L) was added as fungal co-substrate for growth and the pH was adjusted to 4.5	• After glucose addition, COD rose to 2300 mg/L. • MnP activities were of 2.16, 1.92, 0.84 and 0.18 U/L at 48, 72, 96 and 120 h, respectively. Decolorizations were 13% at 24 h, 29% at 48 h, 45% at 96 h and 49% at the end of the experiment. No positive correlation was seen between both parameters. • After ten days, pH was of 7.78, BOD_5 removal of 100%, COD removal of 48%, and an increase of 60 mg biomass, but comparison with unseed controls (where glucose consumption and COD removal were the same than in the seed flasks) indicated no fungal degradation of the leachate. • Competition between the seed fungus and autochthonous microorganisms present in the leachate could be the explanation for these results.
Bardi et al. (2017a)	• Leachate from Brady Road landfill (Winnipeg, Canada), with pH 8.4, 1630 mg/L COD, 940 mg/L NH_4-N and 150 mg/L BOD_5. • *Bjerkandera adusta* MUT 2295, immobilized on polyurethane foam cubes of 2 cm^3 for 7 days in flasks containing glucose and yeast extract broth. • Flasks with 7 cubes and 160 mL leachate, incubated at 25ºC and pH 4.5 for 10 days with shaking (150 rpm) and 0.5, 1.0, 2.5 and 5.0 g/L cellulose as co-substrate. • Flasks with 160 mL effluente (50% diluted or undiluted), 2.5 g/L cellulose and free fungi at the same conditions	• In the flasks with cubes, the greatest MnP activities were reached for 2.5 g/L cellulose (9.6 U/L at 48 h and 26 U/L from 96 to 240 h), whereas 5 g/L gave the highest decolorizations (25% at 24 h and 43% at 240 h). The trend in COD removal was not easily seen because occasional increases of COD due to cellulose solubilisation (total in the flasks with 0.5 and 1 g/L, and incomplete in the flaks with 2.5 and 5 g/L). The biomass did not show a markedly colored aspect after the trials. • In the flasks with cubes, dilution did not affect significantly the MnP activities (which reached maxima at 48 and 182 h). They correlated well with the COD removal in the undiluted leachate, but not with that in the diluted one.

		• Experiments with immobilized fungi and 1 g/L of malt extract or glucose instead of cellulose were performed, but only MnP activities were measured. 1 g/L malt extract gave the highest activities, whereas 1 g/L cellulose gave the lowest ones.
Bardi et al. (2017a)	• Leachate from Brady Road landfill (Winnipeg, Canada), with an average composition of 1585 ±108 mg/L COD, 175 ±35 mg/L BOD_5, 725 ±202 mg/L NH_4-N and pH 8.5 ±0.14 • *Bjerkandera adusta* MUT 2295, immobilized on polyurethane foam cubes as described in the previous row • Two packed bed reactors, with working volumes of 4.5 L, air flow of 2 L/min, a cage (containing 60 foam cubes) fixed to a rotating shaft (5 rpm) and hydraulic retention times of 72 h. Temperature was maintained at 20 – 25ºC and pH to 6. In the first apparatus, cellulose (0.5 g/L) was added in the cage as co-substrate, the leachate was diluted to 33% using deionized water and the operation lasted for 69 days. In the second device, 0.5 g/L glucose were added (in the tank), the dilution was of 50% and the duration of 99 days. An additional co-substrate (0.5 g/L glucose) was put directly in both reactors (after 53 days for the first one and after 75 days for the second one).	• In the first bioreactor, results were divided in three phases. The first one occurred within the first 20 days, and the average COD removal was 11%. The second phase occurred between day 20 and day 50, and the average COD removal was around 15%. In the third phase, the COD removal increased sharply after the addition of glucose, being 54% at day 53 and decreased to 27% at the end of the treatment. • In the second bioreactor, results were divided in three phases as well: from day 1 to day 25, from day 25 to day 60 and from day 60 to day 99. Average COD removals in the three were 51, 14 and 27%, respectively. The decrease of the reactor efficiency in the second phase was not explained. The addition of glucose also caused a improvement of the COD removal, being 44% at day 80. • The authors suggested that the complementary effect of the two co-substrates could be exploited by adding glucose as start-up and cellulose in a second step of the treatment. They proposed non-continuous cosubstrate addition too, for reducing the costs of continuous co-substrate dosage
Awasthi et al. (2017)	• Leachate from the municipal solid waste dumping site Katondha, Jabalpur (India) • Crude enzyme filtrate from *Trichoderma harzianum* (FGCC#A29)	• Previous experiments were done, where the concentration of the leachate varied from 0% v/v to 80% v/v, and temperature from 20 to 60ºC in order to determine suitable experimental conditions, but only enzymatic activities were measured.

	• Flasks with 80% v/v leachate at 45ºC incubated for 168 h. The initial COD in the flasks were of 2358 mg/L	• COD reduction (86.09%) and ecotoxicity reduction (35.6%) were maximum at 120 h. At the end of the experiment, COD removal was of 78.12% and ecotoxicity removal only of 62.6%
Amaral et al. (2017b)	• Landfill leachate from the state of Minas Gerais (Brazil), operating since 2005 with variable concentration. Air stripping was performed in order to decrease its high amounts of NH_4-N. • The yeast *Saccharomyces cerevisiae* • Submerged membrane bioreactor, consisting of hollow fiber membranes of polyvinylidene fluoride, with 0.047 m^2 of area and 0.04 μm of pore size. Experiments were carried out in two phases: in the acclimatization stage, the feed consisted of 3 g/L Sabouraud broth with 20% leachate (days 1 – 30), 40% leachate (days 31 – 48), 60% leachate (days 49 – 59), 80% leachate (days 60 – 69), 100% leachate (days 70 – 79), and after that, the concentration of broth decreased to 1 g/L (days 80 – 91). At day 92, the post-acclimatization stage started, and lasted to day 178. Operational conditions were: infinite sludge age during the acclimatization and 60 days after that, constant permeate flow of 0.3 L/h, air flow rate of 0.6 m/h, pH 3.5, temperature of 25 – 30ºC and hydraulic retention time of 48 h. Backwash was performed every 15 min with duration of 15s, with a flow rate 1.5 times the filtration one. Furthermore, the membrane underwent weekly maintenance cleanings with 500 mg/L NaOCl solution for 20 min in an ultrasonic bath.	• In the acclimatization step, for 20% leachate and 3 g/L broth, the feed contained around 4050 mg/L COD, 550 mg/L DOC, 3365 color units, 100 mg/L TN and 21 mg/L NH_4-N, being their average rejections of 66, 47, 67, 28 and -57%, respectively. For 40% leachate and 3 g/L broth, the feed contained 5900 mg/L COD, 1122 mg/L DOC, 11948 color units, 153 mg/L TN and 32 mg/L NH_4-N, being their rejections of 63, 52, 88, 29 and -12%, respectively. For 60% leachate and 3 g/L broth, the feed contained 6700 mg/L COD, 1525 mg/L DOC, 12581 color units, 255 mg/L TN and 53 mg/L NH_4-N, being their rejections of 60, 53, 86, 26 and -20%, respectively. For 80% leachate and 3 g/L broth, the feed contained 7800 mg/L COD, 1736 mg/L DOC, 14606 color units, 355 mg/L TN and 94 mg/L NH_4-N, being their rejections of 58, 52, 81, 27 and -12%, respectively. For 100% leachate and 3 g/L broth, the feed contained 9493 mg/L COD, 2136 mg/L DOC, 22238 color units, 415 mg/L TN and 94 mg/L NH_4-N, being their rejections of 52, 36, 82, 21 and -12%, respectively. For 100% leachate and 1 g/L broth, the feed contained 8800 mg/L COD, 1666 mg/L DOC, 21814 color units, 396 mg/L TN and 83 mg/L NH_4-N, being their rejections of 72, 51, 83, 37 and 8%, respectively. In the post-acclimatization stage, the average feed concentration was of 9000 mg/L COD, 2374 mg/L DOC, 21204 color units, 343 mg/L TN and 91 mg/L NH_4-N, with rejections of 72, 49, 82, 27 and 24%, respectively. • Toxicity tests with *Vibrio fischeri* were performed. Air-stripping lowered the toxicity of the raw leachate. Dilution with water or with the broth decreased it as well. With 100% leachate and 3 g/L broth, the reduction of the toxicity was around 50%. However, from days 80 to 178, the acidification with H_2SO_4 to keep the pH around 3.5, caused an increase of the toxicity of the feed when compared with the air-stripped

		leachate, and after the treatment, the toxicity of the permeate was around that of the air-stripped stream. • Suspended solids took values of 9700 mg/L at day 1, 4525 mg/L at day 42 and 15350 mg/L after 106 days. Operational problems caused the biological tank sludge to overflow, reducing them to 10000 mg/L at day 115. After that, the concentration slightly increased and remained constant around 11304 mg/L. The mean particle size of the seed yeast sludge was 19.7 μm, decreased to 15.9 μm after feeding with 20% leachate and 3 g/L broth, increased to 25.4 μm with 100% leachate and 3 g/L broth, and fall again to 15.1 μm during the post-acclimatization stage. The higher the particle size, the lower the membrane fouling.. • Concentrations of soluble microbial products were higher than those of extracellular polymeric substances, and both increased along the experiment. The accumulation of these substances on the membrane increased the membrane fouing.
Amaral et al. (2017a)	• Landfill leachate from the municipality of Sabará (Minas Gerais, Brazil), operating since 2005 with BOD_5/COD <0.1 and variable concentrations. Air stripping was performed in order to decrease its high amounts of NH_4-N. • Baker's yeast sludge (*S. cerevisiae*) and activated sludge • Submerged membrane bioreactor, consisting of hollow fiber membranes of polyetherimide, with 0.45 μm of pore size. Filtration areas were 14 m^2 for de bacterial device and 0.04 m^2 for the fungal one. Experiments were carried out in two phases, as in Brito et al.(2012) and Brito (2013). The post-acclimatization stage lasted for 220 days. Operational conditions were: inoculation with 10 g/L suspended solids, infinite sludge age during the acclimatization and 60 days after that, constant	• In the post-acclimatization, for a leachate with 4163 ±828 mg/L COD, 1333 ± 350 color units, 581 ± 310 mg/L NH_4-N and 33.9 ± 8.8 mg/L phosphorum, the yeast bioreactor gave removals of 68 ± 12% COD, 79 ± 8% color, 58 ± 18% NH_4-N and 62 ± 19% phosphorus, whereas, the bacterial bioreactor gave removals of 44 ± 18% COD, 46 ± 20% color, 45 ± 17% NH_4-N and 29 ± 15% phosphorus. • In the yeast bioreactor, the pH was maintained at 3.5 to inhibit the growth of other microorganisms, but filamentous fungi and non-filamentous colonies were detected. The concentrations (in CFU/mL) during the acclimatization phase and the post-acclimatization phase were close to those found in the work of Brito (2013). • For the bacterial bioreactor, weekly chemical cleaning was performed, whereas, for the fungal one, it was fortnightly. Despite this fact, fouling was higher in the bacterial device (average hydraulic permeability of 8.34 $L/m^2 \cdot h \cdot bar$) than in the yeast one (average hydraulic permeability

	permeate flux of 5 L/m^2.h, air flow rate of 0.6 m/h and hydraulic retention time of 48 h. Backwash was performed every 15 min with duration of 15 s. Furthermore, the membranes underwent weekly or fortnightly maintenance cleanings with 500 mg/L NaOCl solution for 4 h followed by a citic acid solution with a pH less than 2 for 20 min.	of 32.23 L/m^2·h·bar). Generation of extracellular polymeric substances and of soluble microbial products was higher for the activated sludge than for the baker's sludge as well.
Reis et al. (2017)	• Leachate from the Macaúbas sanitary landfill, in Sabará (Minas Gerais, Brazil), operating since 2007. After an air-stripping to remove ammonia, its average composition was of 4463 mg/L COD, 1366 color units, 615 mg/L NH_4-N, 1563 mg/L TN, 4121 mg/L Cl and 19 mg/L P. • The same yeast and bacterial reactors of Amaral et al. (2017a) were employed, but with a final nanofiltration unit for further polishing of the effluent. This unit was operated with feed and permeate flow of 144 L/h, pressure of 7.5 bar and recovery rate of 60%.	• In the yeast bioreactor, average removals were of 69 % COD, 54% color, 34% NH_4-N, 34% TN, 50% Cl and -5% P, whereas in the bacterial device, they were of 27% COD, 33% color, 27% NH_4-N, 2% TN, 28% Cl and 7% P. • In the nanofiltration step, these removals were of 93% COD, 94% color, 72% NH_4-N, 59% TN, 82% Cl and 96% P for the fungal apparatus and 86% COD, 100% color, 71% NH_4-N, 59% TN, 77% Cl and 87% P for the bacterial one. • Toxicity was evaluated with *Aliivibrio fischeri*. The bacterial treatment decreased the toxicity of the air-stripped leachate, but the fungal bioreactor increased it. In fact, its permetate was eight times more toxic than the permeate of the reactor seed with activated sludge. After nanofiltration, all the toxicity of the bacterial permeate was removed, but that of the yeast permeate was similar to that of the bacterial one before polishing. • 145 organic pollutants present in the streams were identified by gas chromatography coupled to mass spectrometry. They belonged to carboxylic acids, alcohols, aldehydes, amides, amines, ketones, esters, ethers, phenols, phosphates, hydrocarbons, isocyanates, terpenes and halogenated, among others. 27 of these chemicals only appeared in permeates, so, they were generated during the microbial degradation. The toxicity of these compounds alone do not explain the results, but

the interactions between them may further elucidate the causes of toxicity.