Mycological and ecotoxicological characterisation of landfill leachate before and after traditional treatments

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Abstract

Pollution caused by landfill leachates is one of the main problems of urbanised areas, on account of their chemical composition, which turn in an ineffective treatment. A characterisation of leachates, which takes in to account chemical, ecotoxicological and mycological aspects, is basilar for the evaluation of environmental impact of leachate and the development of suitable treatment techniques. In this study, the toxicity of a raw leachate and an effluent coming from traditional wastewater treatment plant was assessed by means of 4 ecotoxicological assays. Both the samples exceed the legal threshold value according to all the tested organisms, indicating the ineffectiveness of activated sludge treatment in the reduction of toxicity. The autochthonous mycoflora of the two samples was evaluated by filtration. The fungal load was 73 CFU for leachate and 102 CFU for the effluent. Ascomycetes were the dominant fraction (81 % and 61 %, for leachate and effluent respectively), followed by basidiomycetes (19 % and 39 %, respectively). Most of them were potential emerging pathogens. A decolourisation screening with autochthonous fungi was set up towards both samples in the presence or absence of glucose. Eleven fungi (basidiomycetes and ascomycetes) achieved up to 38% decolourisation yields, showing to be promising fungi for the bioremediation of leachates. Further experiment will be aimed to the study of decolourisation mechanism and toxicity reduction.

Key Words: Landfill leachate, autochthonous mycoflora, ecotoxicity, bioremediation.

1 Introduction

Municipal landfills are polluted sites rapidly increased with urbanization and economic development. The percolation of rainwater during the decomposition of wastes generates leachates, which are among the most

polluting wastewaters. The concern about landfill leachates is threefold on account of their difficulty in treatment, their toxic potential and the microbiological composition.

The main difficulties observed during the treatment of leachates under conventional processes are associated with the high concentration of organic matter, the high concentration of nitrogen as ammonia originating from the decomposition of proteins, the presence of toxic organic compounds and heavy metals, and the variable pH values (Vedrenne et al., 2012; Zhao et al., 2013). This is particularly true for mature leachate in methanogenic phase (at least 10 years old), in which the organic matter is principally constituted by recalcitrant and toxic xenobiotics, indicated by a low BOD/COD ratio (<0.5 mg/l), that represents a limit for the growth and the metabolic activity of heterotrophic bacteria in activated sludge (Gotvajn and Zagorc-Koncan, 2009; Renou et al., 2008; Schiopu and Gavrilescu, 2010). The ineffectiveness of traditional treatments is particularly evident in the persistence of the dark colour in effluents coming out from wastewater treatment plants (Kurniawan, et al, 2010; Primo et al., 2012).

Moreover, some recalcitrant compounds are hardly traceable (even in untreated leachate); nevertheless their impact on the environment is potentially devastating on account of their toxicity. The presence of toxic substances can be indirectly detected thanks to ecotoxicity bioassays (Thomas et al., 2009; Tigini et al., 2010). Innovative treatment techniques, effective towards both micro and macro-pollutants, are required.

The biological pollution caused by leachates is another crucial aspect: the microorganisms load due to the presence of high amount of organic matter can pollute both surface and underground waters, with enormous damage to fresh water reserves (Schiopu and Gavrilescu, 2010). Nevertheless, the presence of fungi in leachates has mostly been overlooked (Bareither et al., 2013; Matejczyk et al., 2011; McDonald et al., 2010). A possible reason for this is that the consumption of fungal contaminated water usually does not lead to acute disease, contrarily to pathogenic bacteria, viruses, and parasites contamination (Pereira et al., 2009). Only in the last decade fungi have received increased attention as water contaminants but still few countries have introduced specific law on this aspect (Hageskal et al., 2009).

Thus, a characterisation of leachates, which takes into account chemical, ecotoxicological and microbiological aspects, is basilar for the evaluation of the environmental impact of leachates. In addition, it could be a key element for the assessment of wastewater treatment and, eventually, the development of innovative and more effective techniques. For instance, saprotrophic fungi that access leachates from the landfill waste are potentially suitable microorganisms for the bioremediation of leachate itself. Actually, they are already

adapted to the wastewater toxicity and extreme physical parameters (high ammonium and salt concentration and high pH).

In this preliminary study, a crude leachate and an effluent coming from nitrification-denitrification and biological oxidation treatments were characterised by means of ecotoxicological and mycological analyses. Moreover, the autochthonous fungal stains were tested in a decolourisation screening towards both the raw leachate and the effluent, in presence or absence of an additional carbon source (glucose).

2 Materials and Methods

2.1 Wastewaters

The two samples come from a wastewater treatment plant located in the central part of Italy. One was a crude landfill leachate and the second one was the effluent (consisting of 70 % v/v leachate and 30 % v/v of other kinds of wastewater) previously treated by biological oxidation with activated sludge and nitrification/denitrification process. Both the samples were prepared by sampling the leachate and the effluent daily for a period of 15 days. Both the samples were dark coloured and had high content of ammonium and salts. Details of their chemical characteristics, provided by the owner of the wastewater treatment plant, are shown in Table 1.

Table 1. Samples chemical characterisation.

Parameter	Leachate	Effluent	Legal threshold limit
Cadmium (as Cd)	<0.001 mg l^{-1}	<0.001 mg l^{-1}	0.02 mg l^{-1}
Total chromium (as Cr)	1.20 mg l^{-1}	0.50 mg l^{-1}	2 mg l^{-1}
Nichel (as Ni)	0.30 mg l^{-1}	0.30 mg l^{-1}	2 mg l^{-1}
Lead (as Pb)	0.073 mg l^{-1}	0.082 mg l^{-1}	0.2 mg l^{-1}
Cuprum (as Cu)	0.60 mg l^{-1}	0.15 mg l^{-1}	0.1 mg l^{-1}
Zinc (as Zn)	0.66 mg l^{-1}	1.38 mg l^{-1}	0.5 mg l^{-1}
Total hydrocarbons	<100 mg l^{-1}	598.7 mg l^{-1}	5 mg l^{-1}
Ammonium (as NH_4)	2266.0 mg l^{-1}	408.0 mg l^{-1}	15 mg l^{-1}
Total nitrogen (as N)	2537.9 mg l^{-1}	514.1 mg l^{-1}	20 mg l^{-1}
Sulphates (as SO_4)	46.9 mg l^{-1}	801.7 mg l^{-1}	1000 mg l^{-1}
Chlorides (as Cl^-)	3193 mg l^{-1}	2550 mg l^{-1}	1200 mg l^{-1}

Anionic surfactants (MBAS)	23.07 mg l^{-1}	37.9 mg l^{-1}	2 mg l^{-1}
Non ionic surfactants (PPAS)	23.48 mg l^{-1}	17.2 mg l^{-1}	
Cationic surfactants	<10 mg l^{-1}	<10 mg l^{-1}	
COD (as O_2)	6166 mg l^{-1}	2099 mg l^{-1}	160 mg l^{-1}
BOD_5 (as O_2)	4209 mg l^{-1}	1410 mg l^{-1}	40 mg l^{-1}
pH	8.10	8.40	5.5-9.5
Suspended solids	1064 mg l^{-1}	604 mg l^{-1}	80 mg l^{-1}
Colour	visible at 1:20 dilution	visible at 1:20 dilution	not visible at 1:20 dilution

Both samples exceeded the legal limit for almost 13 parameters, which variation could be due also to the mix of leachate with other influent in wastewater treatment plant, in addition to the performed treatments. Among these, the colour is the most evident. Despite the Italian legal limit is not objectively fixed (the legal threshold is based on the perception of colour at dilution higher that 20:1), the samples colour is very dark and clearly visible even when diluted. The absorption spectra of 1:4 diluted wastewaters are reported in Figure 1.

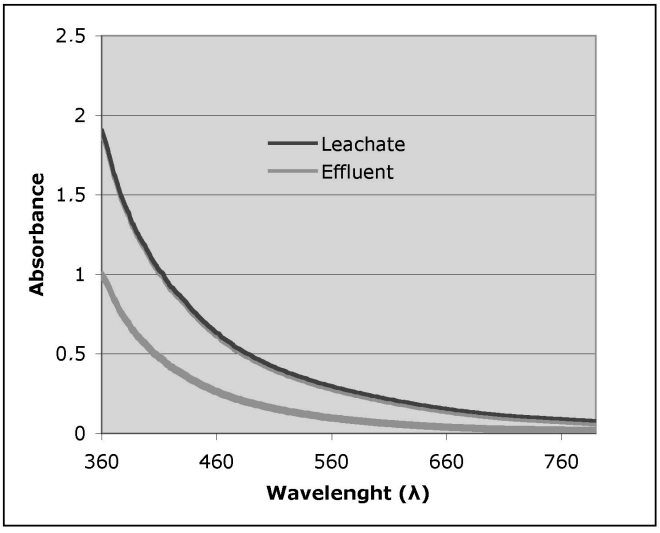


Figure 1. Absorption spectra of 1:4 diluted leachate and effluent.

2.2 Ecotoxicity tests

A battery of four acute ecotoxicity tests was performed in order to evaluate the environmental impact of the leachate and the effluent. The target organisms were selected among different trophic levels: a unicellular green alga (*Pseudokirchneriella subcapitata* (Korshikov) Hindak), two dicotyledonous plants (*Cucumis sativus*

L. and *Lepidium sativum* L.), and a fresh water crustaceous (*Ceriodaphnia dubia* Richard). The endpoints considered were respectively: the algal growth inhibition, the root elongation inhibition, the mobility inhibition. The tests were performed according to the standard methods: UNI EN ISO 8692:2005, EN ISO 20079:2005, UNICHIM N. 1651 (2003), and Ceriodaphtoxkit F (Ecotox, Cornaredo - MI) adhering to the USEPA test guideline, respectively.

After 48 h of incubation at 23 °C and at 8000 lux, the algae concentration was measured with a spectrophotometer. The two dicotyledonous plants, were purchased from Ingegnoli S.p.A. (Milano). For both species, ten seeds were placed in 9 cm Petri dishes, containing 5 ml of sample solution and a paper filter (Whatman No.1). The control was performed in four replicates, using distilled water. The seeds were incubated for 72 h in the dark at 25 °C.

Each dose–response curve consisted of at least 6 dilutions in at least three replicates. To test the sensitivity of each species, acute toxicity tests with $K_2Cr_2O_7$ were performed at regular intervals.

2.5 Isolation and identification of the autoctonous mycoflora

Ten aliquots of 100 ml of each wastewater were filtered on sterile membrane filters (pore size of 0.45 uM) by means of a vacuum pump. The membranes were placed in Petri dishes (9 cm diameter) containing MEA with antibiotics (15 mg l^{-1} streptomycin and 50 mg l^{-1} chloramphenicol). The plates were incubated at 25 °C in the dark. At regular time intervals, the colony forming units (CFU) were counted and the different fungal morphotypes were isolated in pure culture. Fungi were identified conventionally according to their macroscopic and microscopic features. After determination of their genera (Domsch et al., 1980; Kiffer and Morelet, 1997; von Arx 1981), they were transferred to the media recommended by the authors of selected genus monographs for species identification. Molecular identification of all fungi was performed by amplification and sequencing of the internal transcribed spacers (ITS), D1/D2 region, β-tubulin and actin genes (Carbone and Kohn, 1999; Gardes et al., 1993; Glass and Donaldson, 1995; White et al., 1990).

The nonparametric Spearman test was run to assess the significance ($p \leq 0.05$) of the quantitative and qualitative differences between the mycoflora of the two samples.

2.6 Bioremediation screening

Mycelium discs (3 mm diameter) were taken from the marginal portion of colonies in active growth onto MEA and inoculated in multiwell containing 2.5 ml of wastewater samples. The addition of 1 g l^{-1} glucose

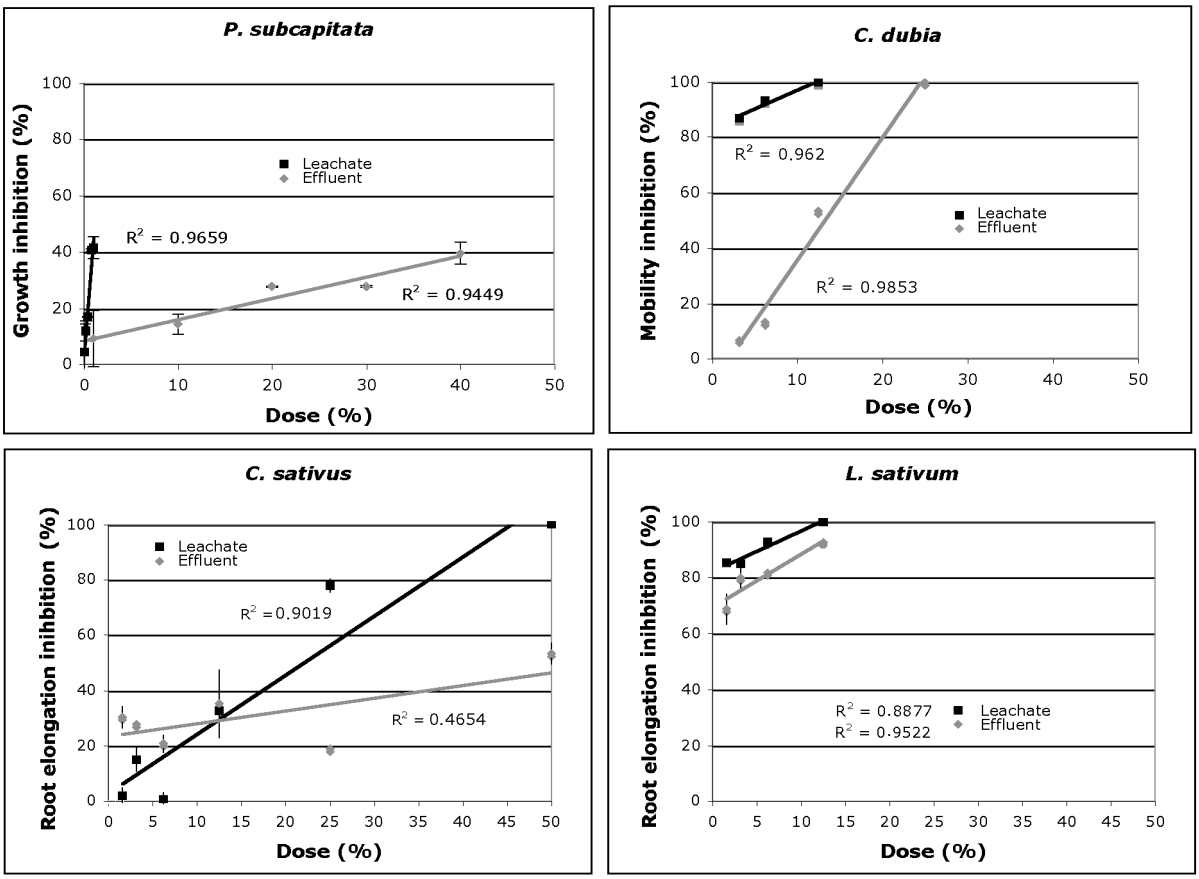
as carbon source was also considered, in order to evaluate a possible co-metabolism. The tests were carried out in duplicate. The multiwell plates were incubated for 20 days at 25 °C, in stirred condition (150 rpm), in the dark. At regular intervals (10-15-20 days) 200 µl were taken from each well, centrifuged at 14000 rpm for 10 minutes, and absorption spectra in the visible (λ = 360-790 nm) were acquired. The decolourisation percentage was determined as the decrease of the spectrum area with respect of the abiotic control.

3 Results and discussion

3.1 Leachate ecotoxicity before and after traditional treatments

A classical elaboration of ecotoxicity results was not always possible. Actually, the calculation of EC_{50}, LD_{50} or LC_{50} was not possible, on account of the sample dark colour that affect the instrumental reading (spectrophotometer). Actually, doses higher than 40% was not testable with the algal test. In other cases, there was a sort of all-nothing effect (*C. dubia* and *L. sativum*), which did not allow to determine a complete dose-effect regression line.

However, all the organisms of the ecotoxicological battery showed clearly a high sensitiveness to the toxicity of the samples. *C. dubia* was the most sensitive organism towards the crude leachate, whereas *L. sativum* was the most sensitive one towards the effluent (Figure 2).



135
136 **Figure 2. Toxicity of leachate and effluent *vs* different test organisms.**

137

138 In general, the leachate was more toxic than the effluent according three out of four tests, as indicated
139 by the regression lines in dose-effect charts of Figure 2. However the toxicity of both samples was much higher
140 than the legal threshold value (DL 152/06), since all the assays showed 100% inhibition at the 100% sample
141 dose (data not shown).

142 The decrease of leachate toxicity after the traditional treatments recorded by *P. subcapitata*, *C. dubia*
143 and *L. sativum* could be due to the lower content of ammonium and, partially, to the decrease of chloride ions
144 with respect to the untreated leachate. On the contrary, sulphate and total hydrocarbon increased in effluent with
145 respect to crude leachate, thus their role in the inhibition of these three organisms may be marginal. In support to
146 this hypothesis, it is noteworthy that as the pH of the samples is adjusted at acidic value (pH 5) the inhibition
147 effect drop under 60% for *L. sativum* (Tigini et al., 2013). Actually, in acidic conditions ammonia (NH_3) turns in
148 the less toxic ammonium ion (NH_4^+). On the base of the same experiment with acidified leachate and effluent,
149 we hypothesized that also heavy metals play a marginal role in samples toxicity. In fact, heavy metals at low pH
150 should be mobilised becoming bioavailable. Despite this, the toxicity decreased as previously reported.

Although for *P. subcapitata*, *C. dubia* and *L. sativum* toxicity could mainly due to the ammonium content in the sample, the synergistic effect of xenobiotics under subacute levels concentration present in the samples can not be excluded. This could be particularly true for *L. sativum*, which recorded a weak decrease of the toxicity after the leachate treatment (Figure 2).

On account of these considerations, the major role of ammonium in sample toxicity could explain the relatively low sensitiveness showed by *P. subcapitata* with respect of other organisms. In fact, the green alga was already reported as a suitable organism for the toxicity assessment of phenyls, aldehydes and alkenes, but not ammonia (Thomas et al., 2009). However, in our case, additional analyses should be performed in order to confirm this hypothesis.

The results obtained by *C. sativus* shows a singular trend, actually crude leachate caused higher inhibition than effluent. However, it is noteworthy that the relationship between effluent dose and *C. sativus* inhibition is not linear, as indicated by the low R value ($R<0.4$). Thus, in this case any comments would be speculative.

3.2 Characterisation of autochthonous mycoflora of leachate

The total fungal load of the crude leachate was very low (7.3 CFU/100 ml) (Table 2).

Table 2. Fungal taxa isolated from leachate and effluent, and their fungal load (CFU / 100 ml^{-1}).

Fungal taxa	**Leachate (CFU /100 ml)**	**Effluent (CFU /100 ml)**
Arthrinium sphaerospermum Fuckel	0.0	0.5
Aspergillus fumigatus Fresen.	0.1	0.0
Aspergillus tubingensis (Mosseray) Kozakiewicz	0.0	0.1
Aspergillus sydowii (Bainier & Sartory) Thom & Church	0.0	0.4
Bjerkandera adusta (Willd.) P. Karst.	0.0	0.1
Candida parapsilosis (Ashford) Langeron & Talice	0.7	0.4
Ceriporia purpurea (Fr.) Donk	0.0	0.1
Coprinellus micaceus (Bull.) Vilgalys, Hopple & Jacq. Johnson	0.2	0.0
Cladosporium brunei Linder	0.0	1.5
Cladosporium cladosporioides (Fresen.) G.A. de Vries	0.4	0.9
Cladosporium perangustum Bensch, Crous & U. Braun	0.0	0.1

Cladosporium pseudocladosporioides Bensch, Crous & U. Braun	2.4	0.0
Cladosporium xylophilum Bensch, Shabunin, Crous & U. Braun	0.5	0.0
Doratomyces microsporus (Sacc.) F.J. Morton & G. Sm.	0.0	0.1
Epicoccum nigrum Link	0.1	0.3
Eurotium amstelodami L. Mangin	0.1	0.0
Flammulina velutipes (Curtis) Singer	0.1	0.0
Galactomyces geotrichum (E.E. Butler & L.J. Petersen) Redhead & Malloch	0.0	0.1
Hamigera striata (Raper & Fennell) Stolk & Samson	0.0	0.1
Hypholoma sublateritium (Fr.) Quél.	0.1	0.3
Leptospheria sp.	0.1	0.0
Penicillium brevicompactum Dierckx	0.5	0.5
Penicillium corylophilus Dierckx	0.0	0.1
Penicillium glandicola (Oudem.) Seifert & Samson	0.3	0.5
Penicillium olsonii Bainier & Sartory	0.1	0.0
Phanerochaete sanguinea (Fr.) Pouzar	0.0	0.1
Phlebia acerina Peck	1.0	0.0
Phlebia tremellosa (Schrad.) Nakasone & Burds.	0.0	0.4
Pseudallescheria boydii (Shear) McGinnis, A.A. Padhye & Ajello	0.6	0.3
Rhodotorula minuta (Saito) F.C. Harrison	0.0	3.0
Talaromyces assiutensis Samson & Abdel-Fattah	0.0	0.1
Talaromyces helicus (Raper & Fennell) C.R. Benj.	0.0	0.2
Total	**7.3**	**10.2**

Despite the high concentration of organic matter in this sample, it is surprising that the total fungal load is similar to that of drinking water (2-9 CFU/100 ml), and even lower than that of ground water (4450 CFU/100 ml) recorded with the same techniques by other authors (Hageskal et al., 2007; Kanzler et al., 2008). This could be due to the high toxicity that affects the sample. In particular, the inhibitory effect of ammonia on fungal growth and enzymes secretion by allochthonous fungi in landfill leachate has been already demonstrated (Ellouze et al., 2009).

The leachate mycoflora consisted of 16 taxa ascribable to 11 ascomycetes, of which 10 filamentous fungi (71 % of the fungal load) and 1 yeast (10 % of total CFU), and 5 basidiomycetes (19 % of total CFU).

178 Most of the ascomycetes were in their anamorphic (asexual) form. They can be considered adaptive
179 extremophiles (Cantrell et al., 2011). Many extremophilic fungal species have a mainly mitotic life style,
180 although even in species with sexual cycles, small isolated populations can diversify independently (i.e.
181 *Eurotium*). This is partially due to the progressing genetic drift rapidly fix alleles in small populations that have
182 managed to adapt to extreme habitats, thanks to the absence of sexuality and/or gene flow (Gostinĉar et al,
183 2010).

184 In addition, some of these fungal species are potentially pathogen (in particular *Candida parapsilosis*
185 which is an H2 organism). The pathogenicity of fungi has been associated with moderate osmotolerance at the
186 order level. Actually, warm-blooded animal body and skin represent an extreme environment for
187 microorganisms, with elevated temperatures and salt concentration, as well as mechanic stress (de Hoog et al.,
188 2005). Thus, generalist fungi with suitable pre-adaptation, as that ones in leachates, represent a pool of potential
189 medically important fungi, which might readily switch from environmental niches to human bodies (Gostinĉar et
190 al., 2010).

191 Opportunistic pathogenic fungi, such as *Aspergillus fumigatus*, *Candida* spp., *Geomyces pannorum*,
192 *Geotrichum candidum*, *Microsporum canis* and *Scedosporium* spp. were often isolates from both polluted water
193 and soil (Tigini et al., 2009; Ulfig, 1994). These fungi are emerging pathogens with an incredibly high ecological
194 success in the last decades. Their rapid spread is particularly alarming on account of their increasing impact on
195 plants, animals and humans (Picco et al., 2011). Many of these species are able to use hydrocarbons, aromatic
196 compounds and natural gas as carbon source; they can growth at very low oxygen partial pressure and in
197 presence of high ammonium and salt concentrations (Kaltseis et al., 2009; Mooner et al., 2013).

198 The presence of basidiomycetes in leachate was notheworty. Actually, they were the second group in
199 terms of both biodiversity and fungal load. Moreover, it must be kept in mind that their biomass can be
200 underestimated considering their CFU, on account of the absence of propagules. These fungi play a key role in
201 the decomposition of organic matter and they represent the last phase of the series that colonise organic matter in
202 the soil (van der Wal et al., 2013). Since their occurrence in aquatic habitats has been reported in several works
203 (Richards et al., 2012), they could play the same ecological role also in these environments, such as leachates.
204 This is supported also by other studies on the composition of fungal community in water contaminated by
205 leachate coming from over than 30 years old landfill, in which basidiomycota was the most represented phylum
206 followed by ascomycota (Brad et al., 2008). Neither in this last study nor in our one the presence of zygomycota

was recorded. Actually, fungi belonging to this phylum are considered pioneers and exploit easy available carbon sources (Anastasi et al., 2013).

3.3 Variation of the mycoflora after traditional treatments

The fungal load significantly increased after the biological oxidation with activated sludge and nitrification/denitrification treatments. However, the effluent showed still a low fungal load (10.2 CFU/100 ml) (Table 2). The biodiversity of the treated leachate consisted of 24 taxa, 16 out of them were not present in the raw leachate. They were ascribable to 18 ascomycetes, among which 17 filamentous fungi (57 % of total CFU) and 1 yeast (4 % of total CFU), and 6 basidiomycetes, among which 5 filamentous fungi (10 % of total CFU) and 1 yeast (29 % of total CFU) (Table 2).

Filamentous ascomycetes remained most abundant group, in particular the anamorphic form.

Yeasts load significantly increased after the leachate treatments. This was due mainly to *Rhodotorula minuta* (basidiomycetes), which was not present in the untreated leachate, and thus it probably came from the treatment plant. This genus seems to be very common in different kinds of wastewater treatment plants (Yang et al., 2011) and represent an emerging halophilic pathogen (Wirth and Goldani, 2012). This yeast can produce cytocrome P450, which can be at the base of several biotransormation and bioremediation process (Fukuda et al., 1996). Its physiological and metabolic characteristics could have determine its acclimatisation in the oxidation tank and, thus, in the effluent.

Filamentous basidiomycetes were halved with respect to the untreated leachate. The turbulent aquatic environment, that generally characterises wastewater treatment plants, is quite unusual for basidiomycetes, on account of their obligate filamentous *habitus* that can be damaged by the continuous stress due to the mechanical agitation. Ascomycetes, instead, can implement an adaptative strategy called microconidiation (microciclic conidiation without the mycelial phase), which allows to overcome this environmental limit. This factor, jointed to the competition with the microflora of the treatment plant, could be the cause of filamentous basidiomycetes decrease. Among the 4 identified basidiomycetes, 3 were exclusively isolated from the treated effluent: *Bjerkandera adusta, Phlebia tremellosa,* and *Phanerochaete sanguinea*. Noteworthy, all of them are well known xenobiotic degraders (Kim et al., 2008; Medvedeva et al., 1994; Spina et al., 2012) and they are probably active in the treatment plant.

3.4 Decolourisation screening

A total of 51 fungi isolated from the raw and treated leachates were deposited in the *Mycotheca Universitatis Taurinensis* (MUT) collection and screened in a decolourisation experiment with the aim to select fungi with high bioremediation potential in terms of decolourisation yields. The addition of 1 g l^{-1} glucose as carbon source was also considered, in order to evaluate a possible co-metabolism of xenobiotics.

Some isolates belonging to the genera *Aspergillus* (*A. fumigatus* MUT 4050, *A. tubingensis* MUT 1288, *A. sydowii* MUT 1290), *Arthrinium* (*A. sphaerospemum* MUT 777), *Penicillium* (*P. brevicompactum* MUT 793, *P. corylophilum* MUT 784), *Pseudallescheria* (*P. boidii* MUT 1269, MUT 721), and some basidiomycetes (*B. adusta* MUT 765, *P. sanguinea* MUT 1284, *F. velutipes* MUT 1275) emerged as the most promising strains. They determined up to 7-26% decolourisation for the leachate and up to 14-23% for the effluent, after 20 days incubation (Figure 3).

The addition of glucose had not determined significant difference in decolouration of wastewaters, indicating a direct metabolism of pollutants. Only *P. sanguinea* MUT 1284 and *P. brevicompactum* MUT 793 showed a significant improvement of the decolouration rate in the presence of glucose (up to 33% decolouration towards leachate and 38% towards effluent, respectively).

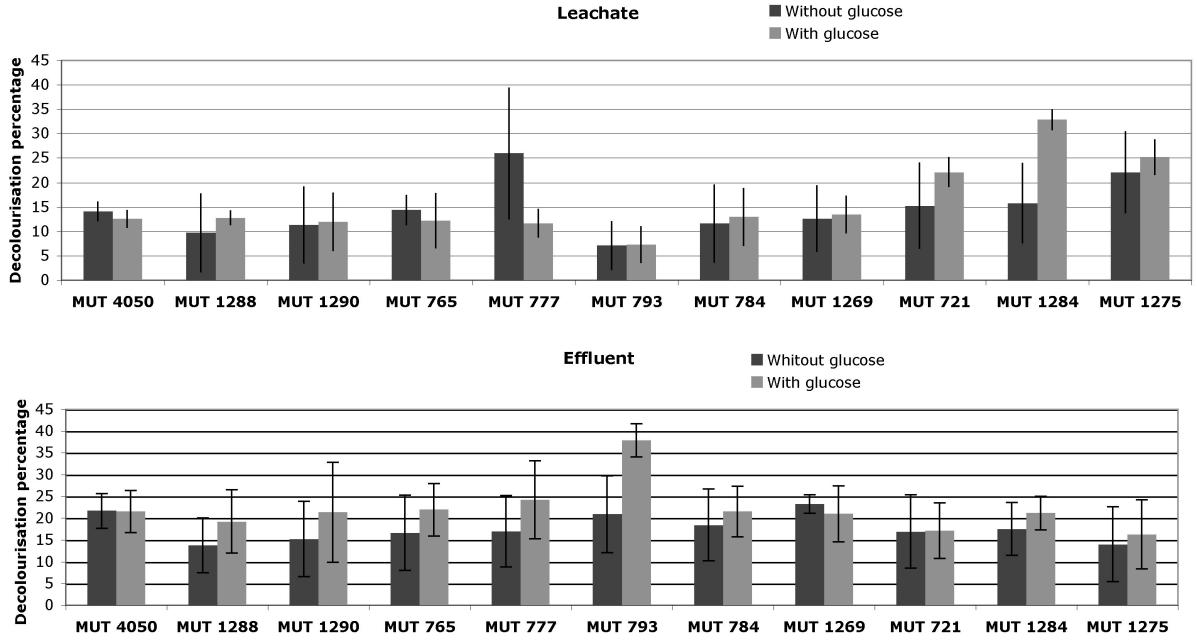


Figure 3. Decolourisation yields of selected autochthonous fungal strains.

Autochthonous basidiomycetes are particularly promising. Actually, the exploitation of an autochthonous fungus for the bioremediation of wastewaters potentially presents a greater rate of success with respect to the allochthonous ones. First of all, strains already adapted to this environment will be more resistant

to the extreme conditions of wastewater. In addition, they can resist also to the competition with other microorganisms present in wastewater (Prigione et al., 2009; Tigini et al., 2009). Thus, the bioaugmentation of an autochthonous fungus in wastewater could bring direct and indirect benefits: the bioremediation of wastewater thanks to its action towards pollutants, and the limitation of pathogenic mycoflora fast and massive consumption of residual carbon source present in it.

In this phase of the research, it was not possible to understand the mechanism at the base of the decolourisation. However, experiments aimed to the discrimination between biosorption and biodegradation processes will be performed in the next future.

4 Conclusion

In our study the high environmental and sanitary risks associated to municipal landfill leachates emerged thanks to an ecotoxicological and mycological characterisation of both a raw landfill leachate and the effluent coming from traditional treatments (both activated sludge and nitrification-denitrification). It can be hypothesised that initial high toxicity of the raw leachate may be the cause of the failure of traditional treatments in the decolourisation. Actually, both the samples exceed the legal threshold value according to all the tested organisms.

Despite the relatively low fungal load, both the samples may represent a risk for human health, on account of the presence of some emerging pathogens. We assessed the possibility to exploit the autochthonous fungi in the decolourisation of the leachate itself, since they are already acclimatised to this extremely toxic environment and are probably able to transform the recalcitrant substances present in it. The results of this preliminary study open interesting perspectives on bioremediation of leachates by means of both biodegradation and biosorption. Further experiment will be aimed to the study of decolourisation mechanism and toxicity reduction.

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