

Detoxification of Tunisian landfill leachates by selected fungi

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Abstract

Young landfill leachates (LFL) collected from Djebel Chekir (Tunisia) discharge area were found to be highly loaded with organic matter, ammonia, salts, heavy metals, phenols and hydrocarbons. Despite the possibility of their biodegradability, they represent a threat to the environment and show some resistance to conventional wastewater treatment processes. For these reasons, this study attempted to develop a biological process for the treatment of LFL using selected strains of *Trametes trogii*, *Phanerochaete chrysosporium*, *Lentinus tigrinus* and *Aspergillus niger*. Experiments were undertaken at different concentrations of the effluent up to 100%. COD removal efficiencies for *P. chrysosporium*, *T. trogii* and *L. tigrinus* were of 68, 79 and 90%, respectively, when LFL underwent a two-fold dilution. COD abatements were accompanied with an important enzyme secretion and a high reduction in the toxicity, expressed as percent bioluminescence inhibition (%BI < 20%). Above 50% of LFL, the effluent was toxic to these strains and caused growth inhibition indicating the sensitivity of these strains to concentrated LFL. Comparatively to the other tested strains, *A. niger* showed to tolerate raw LFL since it grew at 100% of LFL. However, this strain is inefficient in removing phenols and hydrocarbons. Consequently, toxicity abatement was very low (%BI > 70%).

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1. Introduction

In Tunisia, 1700 tonnes of domestic garbage are collected daily [1]. Landfilling is a fundamental step in any waste management strategy. Unfortunately, landfills generate a large amount of leachates containing heavy loads of organics and ammonia nitrogen. These substances can be a major source of contamination to ground water, surface water, fauna and flora. Pumping leachate into wastewater treatment plants may prevent some environmental risks [2]. However, this solution may increase the toxicity of wastewaters since the toxicity of LFL has been reported in several studies [3]. Indeed, the variety of contaminants, their synergistic and antagonistic effects and their physico-chemical properties make of them serious toxicants, which may survive different treatments [3]. For this reason, many strategies of treatment of such effluents have been developed so far. Yet, some persistent drawbacks remain unresolved. Among these

are the complexity of some processes and their expensive costs. These processes, being very often imposed by the characteristics of leachates, varying with the age of landfill, demonstrated high performance [4,5]. Recently, the biological treatments has attracted much attention because of its success in the elimination of non-stabilized organic matter of young LFL and its efficiency in the removal of organics and toxic compounds [6,7].

A good example of this treatment is the use of white rot fungi (WRF) and their enzymes, which showed promising performances in the detoxification of wastewaters and a wide range of xenobiotic environmental pollutants [8,9]. In particular, Abadulla et al. showed that there was a strong correlation between the capacity of WRF to accelerate the biodegradation of organic pollutants and their secretion of extracellular enzymes, such as lignin peroxidases (LiP), manganese peroxidases (MnP) or laccases [10]. The interest in laccases was motivated by their particular capacity to oxidize phenolic and non-phenolic compounds and their ability to decolorize dyes in wastewaters [8,10]. In addition, enzymes of *P. chrysosporium* and *T. versicolor* were found to be very efficient in degrading polyphenols and dyes [11,12]. Finally, D'Souza et al. showed that enzymes of a wide range of WRF could modify lignin [13]. This offers new hopes in finding better lignin degrading enzymes. However, *T. trogii*,

Abbreviations: BI, luminescence inhibition; DM, dry matter; VM, volatile matter; TSS, suspended solids; VSS, volatile suspended solids

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an obvious producer of laccase did not receive much attention. In fact, *T. trogii* has been recently demonstrated to degrade pollutants of high priority, such as polychlorinated biphenyls and polycyclic aromatic compounds [14]. On the other hand, very few reports are available on the ability of *Aspergillus niger* to remove organic pollutants [15]. However, the use of amylolytic fungi for pre-treatment or treatment of wastewaters is a possible option [16,17].

Treatment involving fungi may be beneficial over bacterial treatment since it offers an easier degradation of high molecular-mass organic pollutants (extracellular fungal enzymes) and a higher rate of COD reduction in many problematic wastewaters. However, the use of fungi may include some disadvantages, such as the production of sludge (mycelia) and the requirement of nutrients as well as specific conditions (acidic pH and sterilization), which make the treatment more expensive than bacterial treatment.

The aim of this study is to evaluate the performance of fungal treatment of LFL from Djebel Chekir (Tunisia) and to compare the ability of white rot fungi, such as *P. chrysosporium*, *T. trogii*, *L. tigrinus* to *A. niger* in decreasing the toxicity of leachates.

2. Materials and methods

2.1. Landfill leachate sampling

Raw landfill leachate samples were collected from a landfill site located in Djebel Chekir at 25 km to the south-west of Tunis (Tunisia). If not immediately analysed, samples were stored at 4 °C until use.

2.2. Physical and chemical characterizations

Electric conductivity (EC) and pH were measured using a conductivity-meter (Consort C 831) and a pH-meter (Metrohm), respectively.

Biological oxygen demand (BOD₅) was determined after 5 days by the manometric method with a respirometer [BSB-Controller Model 620 T (WTW)] [18].

COD was estimated as described by Knechtel [19]. Samples were centrifuged at 4000 rpm for 20 min. A total reflux digestion was achieved by reaction with H₂SO₄ and potassium dichromate at 150 °C for 2 h. COD was then determined by OD measuring at 600 nm.

The total nitrogen content (TN) and N–NH₄⁺ were analysed as described in Kjeldahl-N method [20].

Total phenol concentrations were quantified by the colorimetric method [21]. The samples were firstly extracted twice with hexane and methanol (60%, v/v). After 6 h at darkness, the reaction was started after the addition of Folin reactive. The OD was then measured at 727 nm.

Hydrocarbons were determined according to the method described by Marquez-Rocha et al. and adapted to wastewaters [22]. After extraction with the CH₂Cl₂, the organic phase was recovered and dried at a rotate evaporator. Then, the dry weight was measured after stabilisation at 37 °C within 2 h.

Heavy metals concentrations were determined by flame atomic absorption spectrometry of samples digested with an acid mixture of HCl and HNO₃.

For the determination of P-content, samples were digested with HCl and HNO₃. Total phosphorus was determined by colorimetric method as described by Dabin [23].

Volatile fatty acids (VFA) were determined by a gas chromatograph (SHIMADZU) equipped with a flame ionisation detector. A Nukol capillary silica column (30 m × 0.32 mm) was used. Oven, detector and injection port temperatures were 100–150, 250 and 250 °C, respectively. The initial temperature of the column was maintained at 100 °C for 2 min and then the temperature was increased progressively (2 °C min⁻¹) up to 150 °C. One millilitre of mixed liquor sample was centrifuged at 15,000 rpm for 15 min, supernatant was filtered through a MilliPore® membrane of 0.45 μm and 250 μl of filtrate were mixed with 10 μl phosphoric acid (50%, v/v) and 1 μl was injected.

2.3. Strains and culture conditions

2.3.1. White rot fungi strains

T. trogii (CTM 10156) was isolated in our laboratory from a Tunisian biotope [24]. The strain *P. chrysosporium* HD used in this study was a monoconidiosporous isolate from strain BKM-F-1767 (ATCC 24725). *L. tigrinus*, used in this study, was obtained from MUCL 40499. Strains were maintained at 4 °C on malt extract agar medium.

P. chrysosporium and *L. tigrinus* were cultivated on an optimized basal medium containing, per litre, KH₂PO₄ (2 g); CaCl₂·2H₂O (0.14 g); MgSO₄·7H₂O (0.7 g); FeSO₄·7H₂O (0.07 g); ZnSO₄·7H₂O (0.0462 g); MnSO₄·7H₂O (0.035 g); CuSO₄·5H₂O (0.007 g); yeast extract (1 g); thiamine (0.0025 g). The cultural medium was buffered to pH 5.5 with disodium tartrate (10 mM). Veratryl alcohol was added at 0.4 mM. Glycerol served as carbon source at concentration of 10 g l⁻¹. The nitrogen source was the diammonium tartrate (20 mM). Experiments were conducted in duplicate using 100 ml static liquid cultures in 1 l flasks incubated for 15 days at optimal temperature for each fungal strain. All cultures were flushed with pure oxygen. For each fungus, the inoculum was prepared from 5-day-old mycelia malts from agitated or static cultures by washing the mycelia twice with physiological water and fragmented by shaking with glass beads; 2 ml of the suspension (0.3%, v/v) was used to inoculate experimental cultures.

T. trogii was cultivated on a basal medium optimized for the production of laccase. This medium contained (per litre): glucose (10 g); soya peptone (9 g); diammonium tartrate (2 g); KH₂PO₄ (1 g); MgSO₄·7H₂O (0.5 g); KCl (0.5 g); trace elements solution (1 ml). The medium was supplemented with CuSO₄ (0.3 mM) and ethanol (3%, v/v) as inducers of laccases. The cultural medium was buffered to pH 5.5. The shake-flask cultures consisted of 11 Erlenmeyer flasks containing 100 ml of optimized medium supplemented by the inoculum, prepared as was described in *P. chrysosporium* cultures preparation. The inoculated flasks were continuously shaken on a rotary shaking incubator operating at 150 rpm and 30 °C for 15 days. All cul-

tures were supplemented with acidified, sterilized and diluted LFL.

Laccase activity was determined using 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) at a concentration of 10 mM as a substrate according to the method described by Palmieri et al. [25]. MnP was determined according to Paszcynski et al. oxidation assay [26]. The substrate used was the MnSO_4 . LiP was determined using the veratryl alcohol oxidation according to Tien and Kirk [27]. Enzymatic activities were expressed in units per litre of laccase, MnP and LiP.

2.3.2. Culture of *A. niger*

An acid producing filamentous fungus, *A. niger* (An 10) obtained from the Laboratory of Biotechnology at the IRD Centre of Montpellier (France) and isolated in Senegal soil by Dr. Maurice Raimbault, was used in this study. The strain was maintained on potato–dextrose agar slants. Spore suspensions for inoculation were prepared from slants after 5 days of cultivation at 30 °C, using malt extract medium. *A. niger* was cultivated on a basal medium containing, per litre $(\text{NH}_4)_2\text{SO}_4$ (0.5 g); KH_2PO_4 (2 g). The cultural medium was buffered to pH 5.5. The aerobic biological treatment of LFL using *A. niger* was developed and studied in sterile conditions in shake-flask cultures consisting of 11 Erlenmeyer flasks and containing the basal medium supplemented by acidified LFL (pH 5.5). The inoculated flasks were continuously shaken on a rotary shaking incubator operating at 150 rpm and 30 °C for 15 days.

2.3.3. Growth measurements

Growth was measured in terms of dry weight of washed mycelium after filtration and drying overnight at 105 °C on glass-fiber filters (GF/D Whatman Inc.). Yields were expressed as grams of dry weight mycelia per litre of culture.

2.4. Microtoxicity determination

Microtoxicity was carried out with *Vibrio fischeri* (luminescent bacteria LCK 480) using LUMISTox 300 measuring instrument, according to ISO [28]. The inhibition of the bioluminescence of *V. fischeri* using the LUMISTox test kit was achieved by mixing 0.5 ml of LFL and 0.5 ml luminescent bacterial suspension. After a 15-min exposure at 15 °C, the decrease in light emission was measured.

The toxicity of LFL was expressed as percent bioluminescence inhibition (%BI) relative to a non-contaminated reference. A positive control (7.5% NaCl) was included for each test. The results were calculated using a correction factor (C_f), which is the monitor of the changes in intensity within control samples during the 15-min exposure.

2.5. Phytotoxicity

Phytotoxicity was estimated by the determination of the germination index (GI) according to Zucconi et al. method using *Lepidium sativum* seeds [29]. A sample with tap water was used as a control.

3. Results and discussion

3.1. Characterization of the landfill leachate from Djebel Chekir discharge

Four samples were collected at different times of this study. Table 1 presented the average values of different physical and chemical parameters of the collected LFL. LFL has a mean pH of 6.3 and an EC exceeding 20 mS cm^{-1} , showing a high salt level, especially Cl^- and SO_4^{2-} [30]. Taking into account the corrosion problems and the osmotic pressure caused by salts, high salt content must be considered in the design of treatment plant [7]. The BOD_5/COD ratio indicates that the organic matter found in the effluent was biodegradable. However, the content in organic matter largely exceeded 20 g l^{-1} and the BOD_5 value was around 10 g l^{-1} . Im et al. [31] reported a similar composition of LFL with a COD varying from 21 to 26 g l^{-1} and a BOD_5 equal to 10 g l^{-1} . As expected, our analysed samples had high ammonium nitrogen content (728 mg l^{-1}), which represents 80–90% of the total nitrogen contained in the LFL. Many studies focused on the analytical parameters of LFL and found

Table 1
Characterization of raw LFL and Tunisian standards for reject in public canalizations

	Landfill leachate	Tunisian standards of reject
pH	6.3 ± 0.2	6.5–6.8
EC (mS cm^{-1})	29 ± 2	–
Salts (g l^{-1})	24.6 ± 2	–
COD (g l^{-1})	25.2 ± 2.5	1
BOD_5 (g l^{-1})	13 ± 1.5	0.4
BOD_5/COD	0.51 ± 0.05	–
TSS (g l^{-1})	1 ± 0.1	0.03
DM (g l^{-1})	35.5 ± 4	–
VM (g l^{-1})	19.6 ± 2	0.4
VSS (g l^{-1})	0.78 ± 0.06	–
TKN (mg l^{-1})	887 ± 80	100
NH_4^+ (mg l^{-1})	728 ± 60	100
Total phosphorus (g l^{-1})	1.6 ± 0.1	0.01
Total phenols (g l^{-1})	2.84 ± 0.3	0.001
Hydrocarbons (g l^{-1})	3.1 ± 0.3	0.02
Fe (mg l^{-1})	20.6 ± 2	5–15
Pb (mg l^{-1})	0.29 ± 0.02	1
Ni (mg l^{-1})	8 ± 1	1
Cu (mg l^{-1})	1.5 ± 0.2	1
Zn (mg l^{-1})	8 ± 0.8	5
Cr (mg l^{-1})	0.7 ± 0.05	0.5
Cd (mg l^{-1})	<0.2 ± 0.02	0.1
Mn (mg l^{-1})	18.5 ± 2	1
Ca^{2+} (mg l^{-1})	15 ± 1.4	500
Mg^{2+} (mg l^{-1})	32 ± 3	300
Na^+ (mg l^{-1})	59 ± 6	1000
K^+ (mg l^{-1})	9.5 ± 1	50
VFA (g l^{-1})	4.3 ± 0.5	0.03
Acetic acid (mg l^{-1})	1150 ± 100	–
Propionic acid (mg l^{-1})	860 ± 80	–
Isobutyric acid (mg l^{-1})	170 ± 20	–
Butyric acid (mg l^{-1})	1370 ± 150	–
Valeric acid (mg l^{-1})	740 ± 75	–
BI (%)	100 ± 10	–
GI (%)	0	–

similar N–NH₄/total N fraction [3,7,31–34]. LFL samples also contained sodium and potassium but their concentrations did not exceed the Tunisian standards of reject (Table 1).

The mean concentration of VFA was high (4290 mg l⁻¹) with a predominance of acetic and butyric acids (1150 and 1370 mg l⁻¹, respectively) as seen in Table 1. This confirmed that the LFL was young and at least, a part of the organic matter could be degraded biologically. However, phenolic compounds were also present at high amounts (2.8 g l⁻¹). This concentration is not tolerated by Tunisian guidelines of reject into environmental body (0.001 g l⁻¹). Moreover, the hydrocarbons concentrations were also too high (3.1 g l⁻¹) and largely exceeded standards of reject (0.02 g l⁻¹ for Tunisian standards).

LFL was found to be very toxic according to the method using *V. fischeri*, as the most sensitive bacterium. LFL caused 100% of BI of *V. fischeri* (Table 1). This inhibition was still persistent even after an eight-dilution factor (BI > 60%). This is in line with the findings of Marttinen et al. and Silva et al. [3,35], who showed that all samples of LFL exhibited acute toxicity to the bacterium *V. fischeri*. Isidori et al. demonstrated that toxicity increased at higher pH levels and toxic compounds could be characterized as cations, basic chemicals, suspended solids and apolar compounds [2].

LFL was also very toxic to plants since it caused inhibitory effects on the germination seeds and plant growth. Indeed, the GI of *L. sativum* in the presence of LFL was 0% (Table 1). Diluted effluent up to 50 times remained toxic and inhibited seeds germination (the percentage of GI was only 20%).

As a consequence, detoxication of this effluent seems to be imperative prior to its rejection in the sewage or in the environmental body. Fungi and their enzymes were studied for their application in the degradation of problematic pollutants causing environmental problems. Hence, we investigated to test the fungal detoxication of LFL.

3.2. Biological detoxication of LFL by selected fungi

3.2.1. White rot fungi

Treatment assays of LFL using WRF were carried out at different initial CODs (2, 6 and 10 g l⁻¹ for 10, 30 and 50% of diluted LFL, respectively). The selected strains used were *P. chrysosporium*, able to produce MnP and LiP enzymes; *L. tigrinus*, producing laccase and MnP enzymes and *T. trogii*, a strain which produces simultaneously the three enzymes. The three strains tested are known, according to many previous studies, by their degrading ability of problematic compounds that can be present in LFL, such as aromatic compounds. During the treatment assays, these strains strongly decreased the amount of organic matter present in 10 and 30% diluted LFL with an important decrease of pH values (80 and 90% of COD removal, respectively). Using 50% of LFL, the pH decreased progressively from 5.5 to 4.7 for the three species after 7 days of treatment (data not shown). At this concentration of LFL, COD decrease was very important and the abatement values reached 25, 40.4 and 67.5% for *P. chrysosporium*, *T. trogii* and *L. tigrinus*, respectively. These reductions were observed at the third day of treatment. After 7 days of treatment, the COD removal

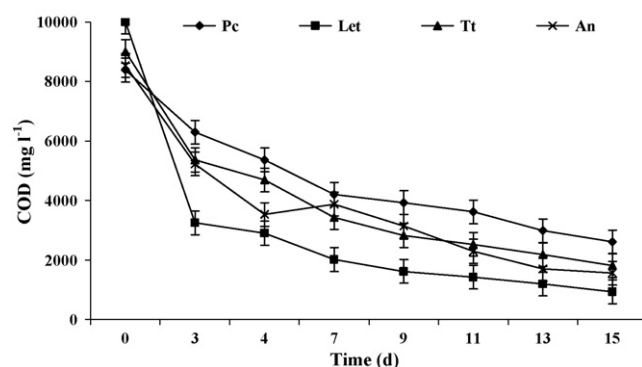


Fig. 1. COD removal during 50% LFL treatment by fungi: (◆) *P. chrysosporium*; (■) *L. tigrinus*; (▲) *T. trogii*; (×) *A. niger*.

efficiencies were of 49.8, 62 and 79.8% for *P. chrysosporium*, *T. trogii* and *L. tigrinus*, respectively (Fig. 1). At the end of the treatment experiments (15 days), 68.8, 79.8 and 90.6% of COD removals were obtained for the three strains, respectively. *L. tigrinus* seems to be more efficient than the two others fungi. In addition, the difference in COD removals observed between *P. chrysosporium* and *T. trogii* was significant. Kim et al. found that *P. chrysosporium* removed only 21.5% of COD and 16.6% of ammonia from landfill leachate pretreated with the natural zeolite clinoptilolite [9].

At the same time, COD removals were correlated with lignolytic enzymes production (Fig. 2). *P. chrysosporium* secreted MnP and LiP at high levels. The maximum production reached 115 and 80 U l⁻¹, respectively (Fig. 2b and c). For *T. trogii*, the production of laccase was high (4000 U l⁻¹) (Fig. 2a). MnP and LiP were also secreted but at low levels (48 and 52 U l⁻¹, respectively) as seen in Fig. 2b and c. In addition, *L. tigrinus* produced high amounts of laccase (980 U l⁻¹), while the amount of MnP produced did not exceed 30 U l⁻¹ as shown in Fig. 2a and b. This may prove that the enzymatic system of these fungi was well involved in the organic compounds degradation. Furthermore, previous works showed that these enzymes contributed to the degradation of aromatic compounds, such as those found in olive mill wastewaters [11].

Fig. 3 shows a high reduction of the effluent toxicity. In fact, the luminescence inhibition of *V. fischeri* strongly decreased during the treatment process and was correlated with the enzymes production and COD decrease. It dropped from 100 to 55% and to 45% since the 4th day for *P. chrysosporium* and *T. trogii*, respectively. With *L. tigrinus*, the BI (%) was less than 20% since the 9th day and therefore, the effluent was considered as non-toxic, as was shown in other studies [36]. With the two others strains, a total removal of the toxicity was also reached and the process decreased the LFL toxicity by more than 80% of the initial value.

This detoxification was a consequence of the important reduction of phenols and hydrocarbons at the end of the treatment. Final phenols and hydrocarbons concentrations obtained, after the treatment by the three strains, were below the standards recommended for reject. In addition, ammonia, which is problematic to microorganisms, was quasi-totally removed by treatment assays for the three strains (Table 2).

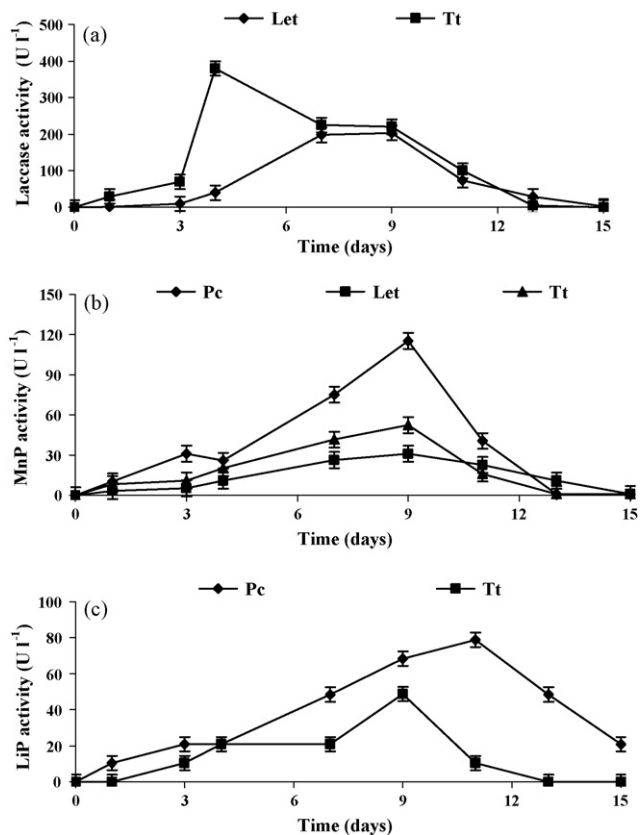


Fig. 2. Enzymes activities during 50% LFL treatment by WRF: laccase (a) (◆) *L. tigrinus*; (■) *T. trogii*; MnP (b) (◆) *P. chrysosporium*; (■) *L. tigrinus*; (▲) *T. trogii* and LiP (c) (◆) *P. chrysosporium*; (■) *T. trogii*.

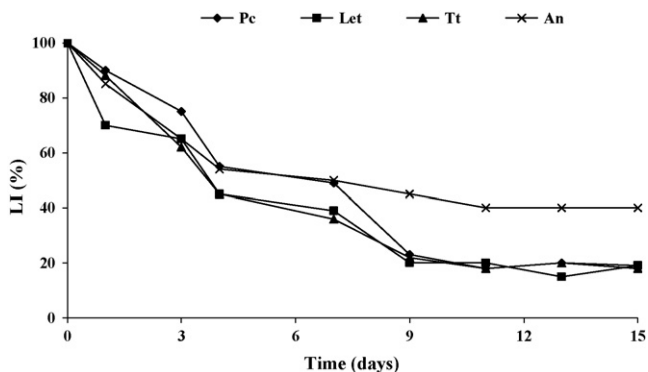


Fig. 3. Toxicity removal during 50% LFL treatment by fungi: (◆) *P. chrysosporium*; (■) *L. tigrinus*; (▲) *T. trogii*; (×) *A. niger*.

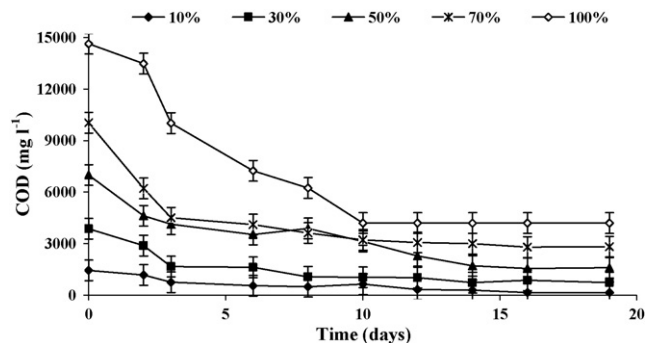


Fig. 4. COD removal during LFL treatment by *A. niger*: (◆) 10%; (■) 30%; (▲) 50%; (×) 70%; (◇) 100%.

3.2.2. Treatment of landfill leachates with *A. niger*

The treatment assays of LFL with pure cultures of *A. niger* were carried out at different concentrations of LFL (from 10 to 100%). The mean pH value of the influent was 6.3. The initial pH values in cultures were experimentally of 5.5, 5.9, 5.9, 5.7 and 5.8 for 10, 30, 50, 70 and 100% of LFL, respectively. The pH values were further reduced approximately to 4.95, 4.25, 5.05, 5.58 and 5.27 after 10 days of treatment for 10, 30, 50, 70 and 100% LFL, respectively (data not shown). This was most likely a result of the activity of *A. niger*. Indeed, previous works showed that this fungus is known for the production of acid metabolite products [37]. This causes a decrease in pH values, which was correlated with an important reduction of the COD values for all the proportions of LFL (Fig. 4). The abatement of COD values reached 77% in the case of 50% LFL (Fig. 1). Besides, VFA were reduced by 82% (data not shown). The highest reduction proportion was observed for acetic acid and butyric acid (89%) (Table 1).

Microscopic observations of cultures showed a biomass constituted mainly of *A. niger* mycelium. At the end of the treatment, the recovered biomass was relatively high for all proportions of LFL. An important biomass growth was observed for 50% LFL (5.8 g l^{-1}). An inhibition effect was observed for 70 and 100% since the quantity of biomass produced was 4 and 2.5 g l^{-1} , respectively. This could be mainly due to the inhibition effects exerted by toxic components in the effluent.

On the other hand, the toxicity reduction by this strain was studied. The influent was toxic since the %BI of *V. fischeri* was 100%. After 3 days of incubation with *A. niger*, only 35% of toxicity reduction was observed (%BI = 65%). At the end of the process, the percentage of toxicity reduction did not exceed 60% for 50% LFL (Fig. 3). The %BI was about 70% for both 70% LFL and 100% LFL (data not shown). This could be due to the

Table 2
Concentrations of ammonia, phenols and hydrocarbons before and after 50% LFL treatment with fungi

Strains	Ammonia (mg l^{-1})		Phenols (g l^{-1})		Hydrocarbons (g l^{-1})	
	NT-LFL	T-LFL	NT-LFL	T-LFL	NT-LFL	T-LFL
<i>P. chrysosporium</i>	728	63	2.84	0	3.1	0.02
<i>T. trogii</i>	728	45	2.84	0	3.1	0
<i>L. tigrinus</i>	728	59	2.84	0	3.1	0.01
<i>A. niger</i>	728	150	2.84	2.5	3.1	2.0

low reduction of phenols and hydrocarbons present in LFL (12 and 36%, respectively). Cereti et al. reported that the reduction of total phenols from olive mill wastewaters by *A. niger* was minimal [38].

Furthermore, the salinity of the influent increased slightly during the treatment with *A. niger*. Nevertheless, this did not seem to have any effect on biodegradation. This finding differs from previous works claiming that high salts concentrations constituted an important limitation for the biological treatment of saline wastewaters [37]. In this study, the salts content of LFL used, as estimated from the non-volatile solids (Table 1), did not exceed 2% (w/v), a level tolerated by a wide range of microorganisms [39].

4. Conclusion

The young LFL, which was investigated in the present work, showed a high level of organic compounds (COD more than 20 g l⁻¹) and toxic effects since the raw effluent completely inhibited both the bioluminescence of *V. fischeri* (BI of 100%) and the germination of *L. sativum* seeds (GI of 0%). These inhibitory effects were, possibly, due to the presence of toxic compounds at high levels, such as N-NH₄⁺, hydrocarbons and phenolic compounds. In addition, this effluent had a high salinity, a mean pH value of 6.3 and contained relatively high concentrations of some heavy metals like Fe, Ni, Zn and Mn, exceeding standards for reject.

The biological detoxification, including three strains of WRF: *P. chrysosporium*, *T. trogii* and *L. tigrinus* was very efficient for 50% diluted LFL. The percentages of toxicity reduction were different for the three strains. Moreover, the reduction of COD was important (68.8, 79.8 and 90.6%, respectively) and was accompanied with an important enzymes secretion by each fungus. The concentrations of N-NH₄⁺, phenols and hydrocarbons as well as toxicity were highly reduced. The percentage of luminescence inhibition of *V. fischeri* dropped down to 20% at the end of the process. An effective detoxification of LFL was obtained with these strains of WRF. Using concentrations of LFL exceeding 50%, the mycelia growth was inhibited and no enzymes activities were detected.

On the other hand, *A. niger* showed to tolerate raw LFL since it grew at 100% of LFL. Indeed, COD was highly reduced (71% with non-diluted LFL). N-NH₄⁺ decreased by 80%. However, the strain seemed to be not efficient in eliminating phenols and hydrocarbons in LFL. The residual concentration of organic compounds after the treatment of raw LFL with *A. niger* exhibited a %BI of 70% after 10 days of incubation.

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