Biosorption with autochthonous and allochthonous fungal biomasses for bioremediation and detoxification of landfill leachate

TITLE: Biosorption with autochthonous and allochthonous fungal biomasses for bioremediation and detoxification of landfill leachate

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Abstract

Landfill leachates are not adequately treated in traditional wastewater treatment plants, on account of their problematic peculiarities: i.e. dark colour, high concentration of recalcitrant pollutants and COD, and high toxicity. In this work, 19 biomasses (15 autochthonous and 4 allochthonous) were exploited in biosorption treatment for the remediation of a leachate (influent) and the effluent coming from the biological oxidation with activated sludge and nitrification-denitrification treatment. The effects of the initial pH, the biomass amount, and the medium for the biomass pre-culture were considered. The best configuration was: pH 5, 5 g L^{-1} biomass cultivated on STY medium. Eventually, the two most effective biomasses, *Cunninghamella bertholletiae* MUT 2861 and *Aspergillus fumigatus* MUT 4050, were used in consecutive 2 h cycles in a batch biosorption experiment. The effectiveness of the treatment decreased in subsequent cycles in terms of decolourisation (31-15%). COD, Cl^-, SO_4^{2-}, total N, and toxicity were removed mainly in the second cycle of treatment (up to -36%, -12%, -15%, -17% and -49%, respectively). The results suggest that the effluent toxicity was basically due to uncoloured substances, which were mainly removed after coloured molecules.

Key Words: Biosorption, Ecotoxicity, Fungi, Landfill Leachate, Bioremediation.

1 Introduction

Landfill leachates are among the most polluting and difficult to treat wastewaters. In fact, besides the high concentration of ammonium and the high pH value, the presence of recalcitrant and toxic xenobiotics generally causes the failure of conventional treatments (Ellouze et al. 2009; Vedrenne et al. 2012). In particular, biological treatments are strongly affected by the low BOD/COD ratio (<0.5), typical of leachates from landfills in methanogenic phase, limiting the growth and the metabolic activity of heterotrophic bacteria in activated sludge (Gotvajn et al. 2009; Kurniawan et al. 2010; Renou et al. 2008; Schiopu and Gravrilescu, 2010). The treatment ineffectiveness turns in the persistence of the dark colour in effluents (Primo et al. 2012). These wastewaters have a deep impact on the water body in which they are discharged (Martínez-Graña et al. 2014). Besides, coloured organic substances reduce the effectiveness of UV disinfection treatments, aimed to the reduction of the sanitary impact of leachates (Zhao et al. 2013). As a consequence, effluents from the treatment of landfill leachates represent a dangerous source of biological pollution, on account of their high microbial load (Matejczyk et al. 2011; McDonald et al. 2010; Tigini et al. 2014).

Many alternative treatments have been explored, such as Fenton's reaction, advanced oxidation processes, etc. (Schiopu and Gravrilescu, 2010). Although some physical and chemical methods are effective in the removal of high strength pollutants, the high cost per volume unit is their major drawback (Bareither et al. 2013; Saetang and Babel, 2012).

Biosorption can represent a valuable tool for the implementation of the wastewater treatment, on account of the wide range of target molecules, the cost-effectiveness and, in the case of dead biomasses, the irrelevance of the leachate toxicity (Mishra et al. 2016; Chen et al. 2013). Among different biosorbents, fungal biomasses are of particular interest for pollutant biosorption, thanks to the variety of structural components of their cell wall that ensures many different functional groups, which bind molecules to varying degrees (Gadd, 2009; Tigini et al. 2012). Moreover, fungal byproduct biomasses could present several advantages with respect to other biomasses, since they are abundantly available from the fermentation industry (Prigione et al. 2012).

In the present work, a total of 19 fungal biomasses were exploited in decolourisation experiments for the treatment of two real samples: a crude landfill leachate and the effluent coming from a traditional wastewater treatment plant (nitrification-denitrification treatment and oxidation by means of activated sludge). Four out of 19 were allochthonous fungal biomasses, selected for their biosorption capability (Tigini et al. 2012, 2011) or for their availability as industrial byproducts. The other 15 biomasses originated from 7 autochthonous fungi selected in a previous decolourisation screening (Selbmann et al. 2013; Tigini et al. 2014). The effects of the initial pH, the biomass amount, and the medium for the biomass pre-culture were considered. Eventually, the experimental design (Sup 1) allowed the selection of the two most effective biomasses destined to a biosorption treatment performed in consecutive cycles. The effectiveness of the treatment was evaluated as the removal of colour, COD, Cl^-, SO_4^{2-}, total N, and toxicity.

2 Materials and Methods

2.1 Wastewaters

The two samples came from a wastewater treatment plant located in Italy. One was the influent (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 and nitrification-denitrification treatments. Both the samples were composite mixtures, consisting of the wastewaters daily sampled for a period of 15 days. Both the samples were dark coloured and had a high content of ammonium and salts. Details of their chemical features, provided by the owner of the wastewater treatment plant, were already described in a previous publication

(Tigini et al. 2014). All parameters exceeding the legal threshold values are reported in the supplementary material (Sup 2).

2.2 Allochthonous biomasses

Two byproduct biomasses, biomass A and biomass W, were obtained from ACS Dobfar spa (Tribiano, Italy) and Wetlands Engineering sprl (Louvain La Neuve, Belgium), respectively. The biomass A came from a pharmaceutical fermentation process aimed to the production of antibiotics. The biomass W one is a mix of different fungal biomasses coming from several fermentation processes.

Moreover, *Cunninghamella bertholletiae* Stadel (MUT 2861) (previously cited as *Cunninghamella elegans* Lendner MUT 2861, and recently renamed after been subjected to molecular analyses) was obtained from the *Mycotheca Universitatis Taurinensis* (MUT, University of Turin, Department of Life Sciences and Systems Biology). This strain was selected and patented for its capability to adsorb organic and inorganic pollutants (Tigini et al. 2012, 2011). The fungus was inoculated as a conidial suspension ($1 \cdot 10^5$ conidia mL^{-1}) in an optimised STY medium (20 g L^{-1} potato starch, 20 g L^{-1} double tomato concentrate, 5 g L^{-1} yeast extract), developed by Actygea srl (Puracqua project supported by Lombardia Region, Italy). The biomass was grown for 7 days in stirred conditions (130 rpm) at 30 °C, then it was sieved (150 µm pore) and rinsed several times with water to minimise the residual medium. Then, it was inactivated by autoclaving at 121 °C for 30 min, and collected in non-sterile conditions. Besides, a part of MUT 2861 biomass was lyophilised, as already described (Tigini et al. 2011).

2.3 Autochthonous biomasses

Seven out of fifty-one autochthonous fungi were selected from a miniaturised screening among the most effective in the colour removal from the leachate and the effluent (Tigini et al. 2014). The strains were *A. fumigatus* MUT 4050, *A. tubingensis* MUT 1288, *A. sydowii* MUT 1290, *Arthrinium sphaerospermum* MUT 777, *Penicillium brevicompactum* MUT 793, *P. corylophilum* MUT 784, and *Bjerkandera adusta* MUT 765. They were cultivated in three different liquid media: CSL (20 g L^{-1} Corn Steep Liquor), STY, and EQ (20 g L^{-1} glucose, 2 g L^{-1} ammonium tartrate, 2 g L^{-1} KH_2PO_4, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.1 g L^{-1} $CaCl_2 \cdot 2H_2O$, 10 mL mineral stock solution), to assess the effect of the medium composition on the biosorption effectiveness. The biomasses were grown for 7 days in stirred conditions (130 rpm) at 30 °C. Then, they were sieved (150 µm pore)

and rinsed several times with water to minimise the residual medium, inactivated by autoclaving at 121 °C for 30 min, and collected in non-sterile conditions.

2.4 Biosorption experiments

2.4.1 First step: selection of suitable pH with allochthonous biomasses

Each allochthonous biomass was weighed and an amount of biomass was placed in 50 mL Erlenmayer flasks containing 30 mL of wastewater to reach 10 g L^{-1} ratio (dry weight). Both wastewater samples were used as such (pH 8.5) and after pH modification by means of hydrochloric acid (pH 7, pH 6, pH 5, pH 3). The flasks were incubated at 25 °C in stirred conditions (130 rpm). Each trial was performed in triplicate. Samples without biomass were used as abiotic controls to assess the decolourisation due to other causes than biosorption (e.g. photobleaching or complexation).

At regular intervals, 300 µL of sample were taken from each flask, centrifuged at 14,000 rpm for 5 min, to remove mycelial fragments, and examined with a spectrophotometer TECAN Infinite M200 (Austria) to acquire the absorbance spectrum from 360 to 790 nm. The decolourisation percentage (DP) was calculated as the extent of decrease of the spectrum area, with respect to that of the abiotic control. The significance of differences ($P \leq 0.05$) among DP values achieved by different strains was calculated by the Mann-Whitney test (SPSS inc., 2000).

2.4.2 Second step: selection of suitable biomass/wastewater ratio with two allochthonous biomasses

Two allochthonous biomasses, selected in the previous step, were used in four different ratios of biomass dry weight per wastewater volume: 10 g L^{-1}, 5 g L^{-1}, 2.5 g L^{-1} and 1 g L^{-1}. The biomasses were placed in 50 mL Erlenmayer flasks containing 30 mL of wastewater samples at the pH selected in the previous step. Then, the experiments and the elaboration of the obtained data were performed as previously described.

2.4.3 Third step: selection of suitable biomass pre-culture with autochthonous biomasses

Each autochthonous biomass (in biomass /wastewater ratio selected in the previous step) was placed in 50 mL Erlenmayer flasks containing 30 mL of wastewater, at initial pH selected in the first step. Then the experiments and the elaboration of the obtained data were performed as previously described.

2.4.4 Fourth step: biomass reuse in consecutive biosorption cycles

The two best biomasses, one among the autochthonous and one among the allochthonous, were placed in 30 mL of wastewater, at initial pH selected in the first step, and with the biomass/wastewater ratio selected in the second step. The incubation and the monitoring of the DP were performed as previously described, for a period of 2 hours for each cycles. At the end of each cycle, the content of the flasks was centrifuged and the supernatant was replaced with a new wastewater to be treated. The experiment ended after the third cycle. At the end of the experiment, the treated supernatants and the untreated controls were subjected to ecotoxicity and chemical analyses.

2.4.5 Ecotoxicity and chemical analyses

Before and after the biosorption treatment, the sample ecotoxicity was evaluated by means of the test with the alga *Raphidocelis subcapitata* (Korshikov) Nygoard, Komárek, J. Kristiansen & O.M. Skulberg (UNI EN ISO 8692:2005). This target organism was selected as the most sensitive one towards this kind of wastewaters (Tigini et al. 2014). The COD, Cl^-, SO_4^{2-}, and total N, were determined using Hach-Lange's cuvettes, after sample filtration (filter 0.45 μm).

3 Results and Discussion

3.1 Effect of pH and selection of allochthonous biomasses

The decolourisation percentages achieved by the biomasses (10 g L^{-1}) at different initial pH values are reported in Figure 1.

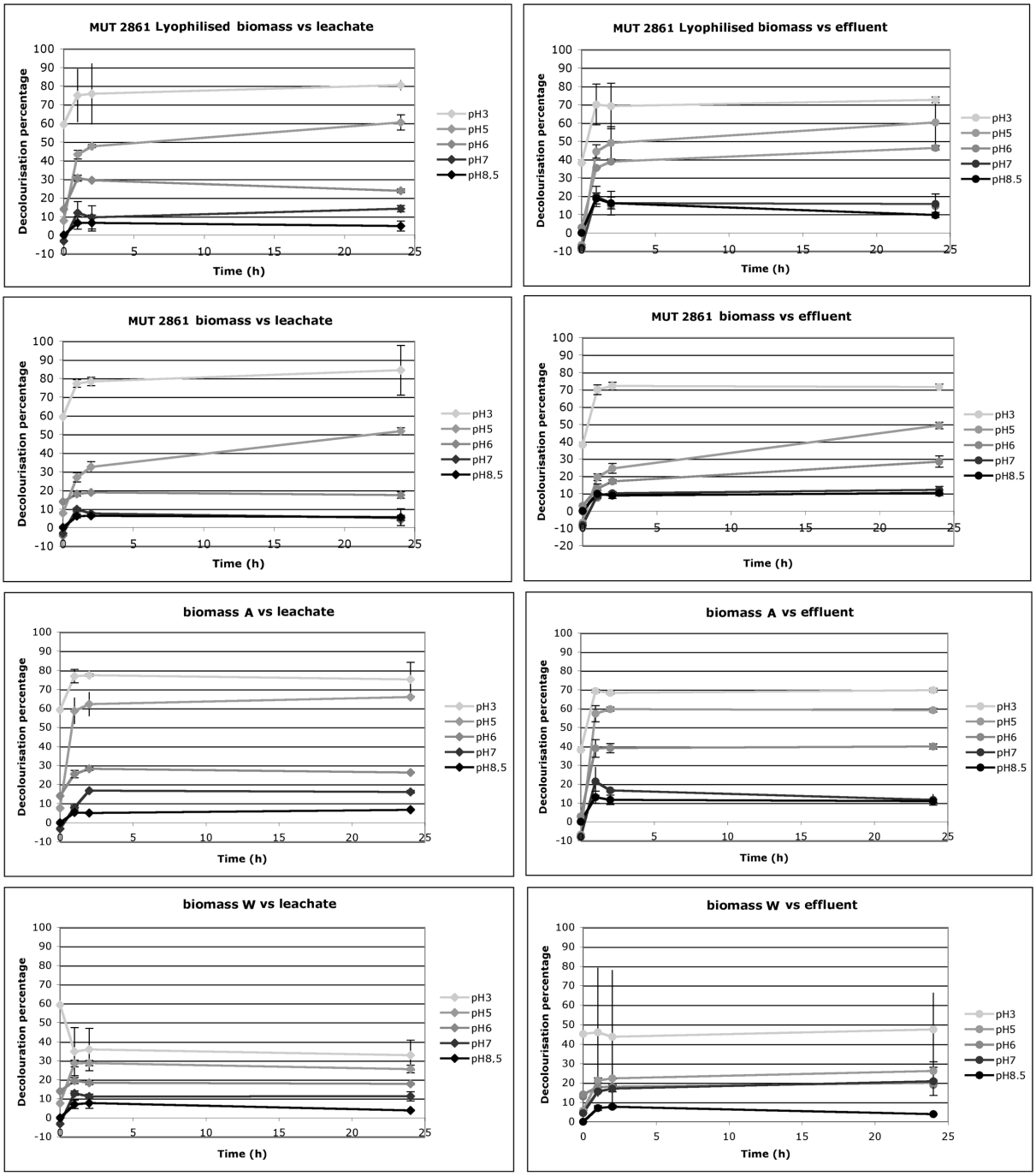

Figure 1. Effect of pH on the decolourisation process by means of fungal byproduct biomasses (A and W) and MUT 2861 biomasses. The decolourisation obtained at time 0 is due to the pH adjustment.

The treatment (pH adjustment followed by biosorption) caused an overall decolourisation ranging between 5% and 80% with respect to the unmodified wastewaters, with the best results obtained at pH 3 (Figure

1). In particular the effluent treated with lyophilised *C. bertholletiae* MUT 2861 biomass complied the Italian legal limit for the colour (not visible at 1:20 dilution).

The pH adjustment brought to the precipitation of a part of coloured substances present in both samples, causing a decolourisation proportional to the acidification of the samples, up to 59% and 38% (DP values at 0 h in Figure 1) for the leachate and the effluent at pH 3, respectively.

The pH strongly affected the biosorption process, too. The decolourisation due to biosorption onto the biomass was generally proportional to the sample acidification up to pH 5, than it decreased at pH 3 (Table 1).

Table 1. Decolourisation percentages of the samples due to biosorption treatment (the values are normalised accordingly to the decolourisation achieved after the pH modification at 0h).

		Leachate				Effluent			
		0 hour	1 hour	2 hours	24 hours	0 hour	1 hour	2 hours	24 hours
MUT 2861 biomass	pH 3	0,0	18.0	19.1	25.2	0.0	31.9	34.0	33.4
	pH 5	0.0	19.4	25.0	44.0	0.0	26.2	31.2	56.0
	pH 6	0.0	4.1	5.0	3.5	0.0	10.0	14.1	25.5
	pH 7	0.0	13.1	10.8	8.4	0.0	16.9	18.1	20.2
	pH 8.5	0.0	6.1	6.2	5.5	0.0	9.9	8.8	10.3
MUT 2861 lyophilised biomass	pH 3	0.0	15.7	16.5	21.2	0.0	32.0	31.1	34.5
	pH 5	0.0	35.7	40.1	52.8	0.0	51.1	55.8	66.9
	pH 6	0.0	16.7	15.5	9.9	0.0	32.5	36.0	43.5
	pH 7	0.0	15.2	12.7	17.4	0.0	27.8	24.2	23.7
	pH 8.5	0.0	6.5	6.5	4.9	0.0	18.7	16.1	9.6
Biomass A	pH 3	0.0	17.7	18.3	16.1	0.0	31.1	30.2	31.6
	pH 5	0.0	51.1	54.6	58.4	0.0	64.0	66.4	65.8
	pH 6	0.0	11.5	14.3	12.4	0.0	36.1	36.2	37.0
	pH 7	0.0	11.1	19.92	19.3	0.0	29.6	24.7	19.8
	pH 8.5	0.0	5.3	5.1	6.7	0.0	13.0	11.6	11.0
Biomass W	pH 3	0.0	-24.5	-23.4	-26.4	0.0	0.7	-1.5	2.2
	pH 5	0.0	21.0	21.2	18.0	0.0	15.6	16.7	20.5

	pH 6	0.0	5.6	4.5	3.9	0.00	3.72	3.94	5.92
	pH 7	0.0	16.1	14.4	14.5	0.0	11.2	12.6	16.4
	pH 8.5	0.0	7.0	7.7	3.9	0.0	1.7	0.8	3.4

Hence pH 5 was selected for the further experiment on biomass/wastewater volume ratio.

The biomass of *C. bertholletiae* MUT 2861 confirmed its high potential in the wastewater decolourisation by means of biosorption (up to 56% decolourisation for the leachate and 44% for the effluent, Table 1). The lyophilisation did not significantly increase the decolourisation at the end of the experiment. Nevertheless, it improved the decolourisation yields during the first 2 hours, in particular for initial pH 5 and 6 (Table 1). The same result was already observed towards textile wastewaters, however in that case the effect of the biomass lyophilisation was more evident (Tigini et al. 2011). Since any biomass pre-treatment causes costs increase, this should be motivated by a significant improvement of biosorption yields (Gadd, 2009). In the present work, the decolourisation yields did not justify the cost of the biomass lyophilisation.

The byproduct biomasses gave opposite results. Biomass W was quite always ineffective, and caused the increase of absorbance spectrum of leachate at pH 3 (Figure 1). Probably, in this case the solubilisation of coloured substances occurred after the contact with biomass W. An optimisation of biosorption parameters, such as physical and chemical pre-treatments of the biomass, could improve the biosorption effectiveness. However, it is important to underline that the advantage associated to the use of a low cost industrial biomass is maintained when the biosorbent is ready to use (Gadd, 2009). Thus, the financial impact of a biomass pre-treatment on the biosorption treatment should be deeply investigated.

On the contrary, the biomass A was very effective in the colour removal (up to 66%) from both samples. This biomass showed to be competitive with respect to the selected strain MUT 2861 in terms of colour removal, as already shown with textile wastewaters (Prigione et al. 2012). The exploitation of byproduct biomasses in wastewater remediation represents a double advantage: it gives new life to a waste, which has high disposal costs, and wastewaters can be properly treated. Moreover, the use of byproduct biomasses is a prerequisite for a financially sustainable process (Michalak et al. 2013). The critical point is to find an industrial process, which continuously generates byproduct biomasses, independently from the seasonality of the market. The pharmaceutical industry reasonably ensure a continuous production.

On account of the present results, untreated MUT 2861 biomass and the biomass A were selected for the further experiment, aimed to the optimisation of biomass/wastewater volume ratio.

3.2 Effect of biomass/wastewater ratio

Decolourisation yields were generally proportional to the biomass amount (Figure 2).

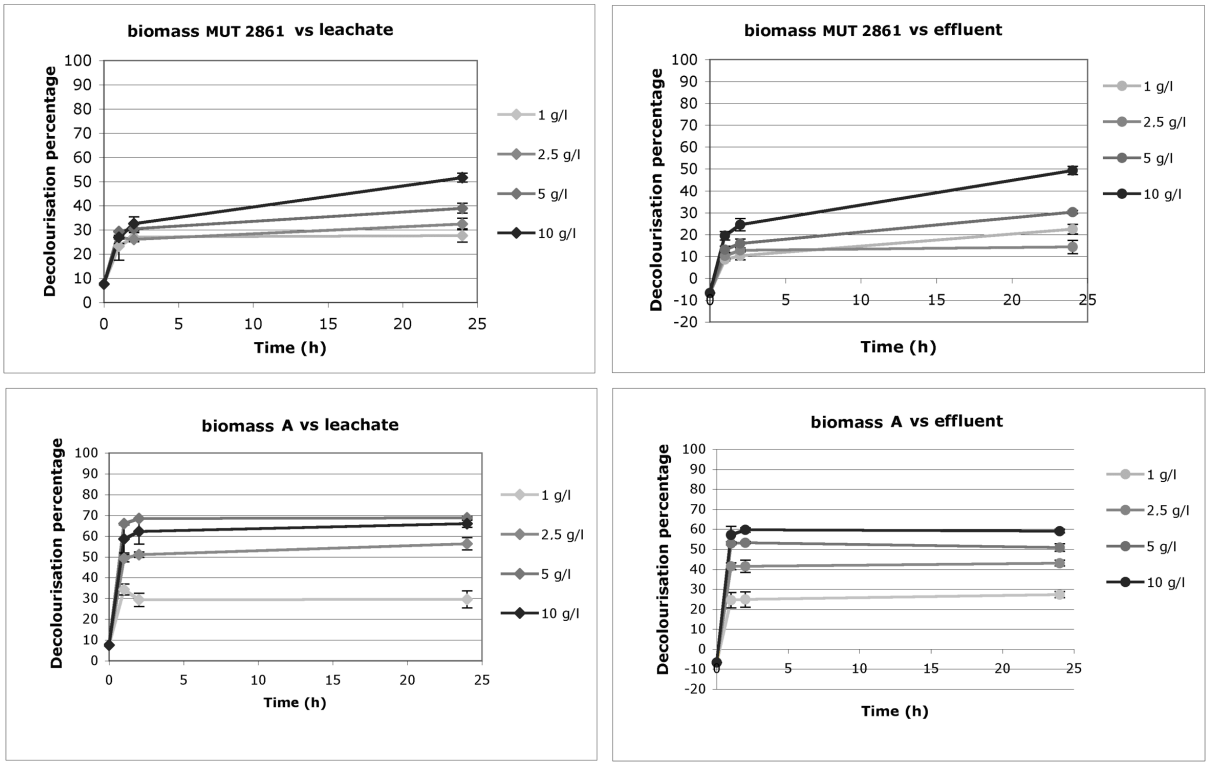

Fig. 2 Decolourisation obtained by different amounts of biomasses A and MUT 2861 towards the unmodified wastewaters. The decolourisation obtained at time 0 is due to the pH adjustment

The biomass A quite always obtained the best result with respect to *C. bertholletiae* MUT 2861 at the same ratio. It should be noted that biomass A has an excellent surface/volume ratio, on account of its creamy texture. Instead, MUT 2861 biomass has a more compact structure. As a consequence, only the outer part of the biomass was in direct contact with the wastewater; whereas, the inner part of the biomass probably never came into contact with the wastewater. In fact, at the end of the treatment, *C. bertholletiae* MUT 2861 showed not coloured areas. This hypothesis can be confirmed by the fast achievement the biosorpton equilibrium by the biomass A (no significantly difference among decolourisation percentage at 1, 2 and 24 h). On the contrary, *C. bertholletiae* MUT 2861 continued to absorb coloured substances, meanwhile the wastewater was permeating the biomass granules (Figure 2).

On the other hand, the effectiveness of the biomass A is to be balance with some drawbacks in the solid-liquid separation phase. The extremely fine texture of the biomass A implies a centrifugation after the treatment, whereas the biomass of *C. bertholletiae* MUT 2861 spontaneously settles within few minutes. This last feature can help in the scale-up of the process at industrial level indeed. Moreover, the exploitation in a repeated long-term application of a biomass, which remains suspended in the wastewater, is not commercially attractive, requiring a continuous biomass consume and additional treatments for its removal from the treated effluent (Gadd, 2009; Liu and Liu, 2008). On account of that, *C. bertholletiae* MUT 2861 was selected for the biosorption process in subsequent cycles experiment.

Since it is important to mediate between the decolourisation yields and the amount of biomass, in order to obtain an effective treatment with a limited production of exhausted biomass, the biomass ratio selected for the further experiment was 5 g L^{-1}. This biomass ratio did not show significant differences, with respect to 10 g L^{-1} ratio, in decolouration yields within 2 h treatment, which is a reasonable duration for biosorption treatment. Actually, DP was not proportionally improved by doubling the biomass amount. Moreover, 5 g L^{-1} is a biomass ratio comparable to other biosorption experiments in literature (El-Sayed, 2013; Jianget al. 2013).

3.3 Autochthonous strains: effect of medium for cultivation and biosorption capability

STY was generally the most productive medium (up to 5.4 g L^{-1} for MUT 1288), whereas EQ was the less productive one (up to 3.1 g L^{-1} for MUT 1288). The only exception was recorded with *A. fumigatus* MUT 4050, for which CSL was the most productive medium (4.8 g L^{-1}), whereas STY was the less productive one (3.4 g L^{-1}). Biomasses with a production lower than 0.5 g L^{-1} were not used for biosorption experiment (Figure 3).

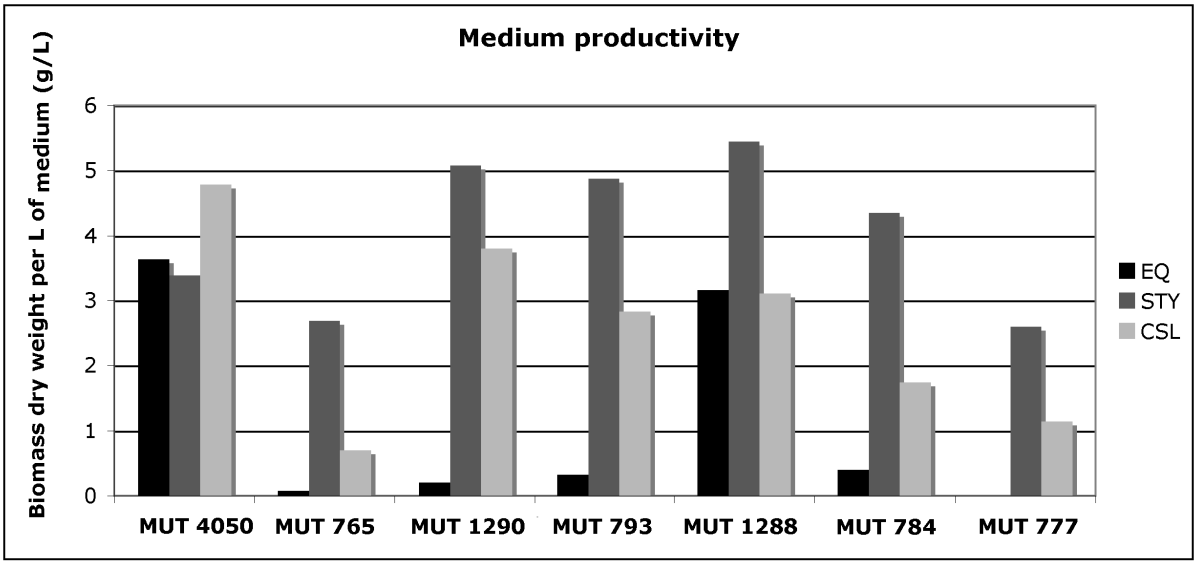

Fig. 3 Productivity of different media for the cultivation of autochthonous fungi

Thus, only 15 biomasses were used (5 g/L ratio) among the 21 overall produced. The results are reported in Figure 4. The culture medium significantly affected the decolourisation yields, when comparisons could be done (only one medium was used for MUT 765). Towards the leachate, 4 out 6 strains achieved the best results when cultivated in CSL medium (DP up to 51% for MUT 1290), with respect to the other culture media. The biomass of *A. fumigatus* MUT 4050 achieved the best result with STY (55% decolourisation), whereas biosorption effectiveness of MUT 777 was not affected by the culture medium (52% decolourisation with both CSL and STY) (Tigini et al. 2012).

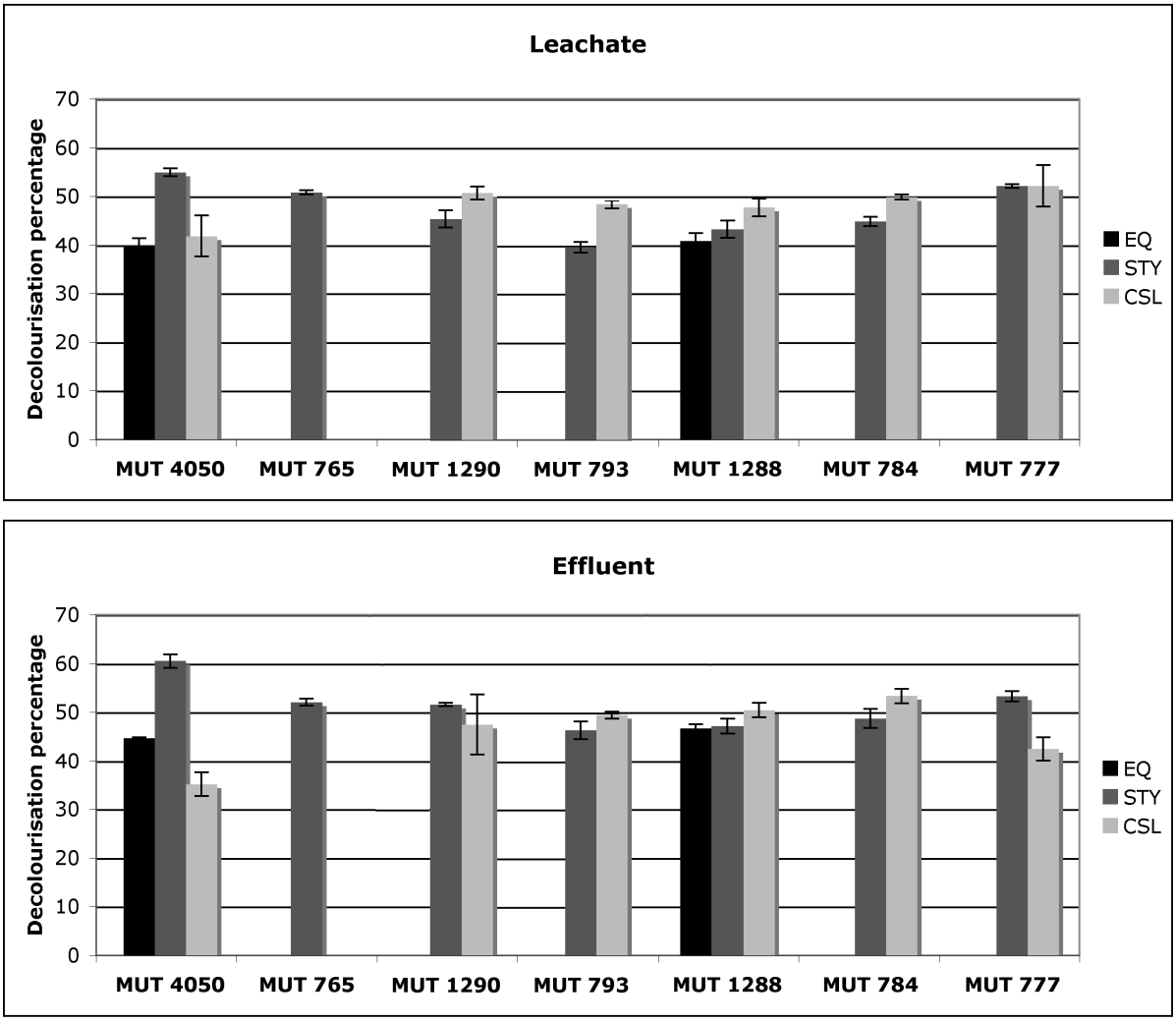

Fig. 4 Decolourisation after 24 h treatment with autochthonous biomasses pre-cultivated in different media

The decolourisation yield towards the effluent followed a similar trend, with comparable DP. Three out of 6 strains achieved the best results when cultivated in CSL medium (up to 53% for MUT 784). *A. fumigatus* MUT 4050 and *A. sphaerospermum* MUT 777 achieved the best results with STY (up to 61%). Whereas, biosorption effectiveness of *A. sydowii* MUT 1290 was not significantly affected by the medium for biomass pre-culture. This is probably due to the fact that coloured molecules present in the leachate and the effluent similarly interact with the biomass functional groups. Thus, this indirectly indicates the recalcitrance of the leachate coloured molecules, which remains in the effluent, without changing their characteristics, after the activated sludge and nitrification-denitrification treatments.

Unfortunately, the reason of the difference in biosorption yields is not clearly explainable, for the lack of information about both the biosorption mechanism and the biomass composition. Starch and CSL are known to influence the polysaccharides amount in *C. berthollethiae* cell wall (Tigini et al. 2012). If these considerations could be true also for the tested autochthonous fungi, it can be hypothesized that for the strains MUT 4050 and, partially, MUT 777, the main cell wall components involved in biosorption process were polysaccharides, which are enhanced by starch in culture medium. On the contrary, for MUT 1290, MUT 793, MUT 1288 and MUT 784, different functional groups could be involved, since CSL decreases acidic polysaccharides in *C. berthollethiae* cell wall (Tigini et al. 2012). Further experiments on the biomass composition of these autochthonous fungal strains are needed to confirm this hypothesis.

Independently from the mechanism involved, the best DP was obtained by *A. fumigatus* MUT 4050 biomass cultivated on STY. On the base of this result, this biomass was selected for the further part of the research. This fungal species is known to have good decolourisation capabilities though both biosorption (Kalaiarasi and al., 2012) and biodegradation (Karim et al., 2017) mechanisms and was patented previously (JPH0639392A). Moreover, since there was no significant difference between decolourisation percentage at 2 h and 24 h (data not shown), the further experiment was performed with 2 h cycles.

3.4 Biomass reuse in consecutive biosorption cycles

The decolourisation yields achieved by 5 g/L of allochthonous (MUT 2861) and autochthonous (MUT 4050) biomasses selected in the previous experiments were comparable for all the experiment. After the first 2 h cycle the biomasses achieved 28-31% decolourisation of the effluent. In subsequent cycles, these biomasses halved their effectiveness, achieving up to 15-18% decolourisation of the effluent at pH 5 (Table 2).

Table 2. Chemical-physical and ecotoxicological parameters and their variation after biosorption treatment cycles with respect to the untreated effluent at pH 5.

		MUT 2861		MUT 4050	
Parameter	Cycle	value at the end of the cycle	Δ with respect to the control at T0	value at the end of the cycle	Δ with respect to the control at T0
Colour	I	-	-31%	-	-28%
	II	-	-18%	-	-16%
	III	-	-15%	-	-15%
COD	I	1925 mg L^{-1}	-24%	1925 mg L^{-1}	-24%
	II	1835 mg L^{-1}	-28%	1610 mg L^{-1}	-36%
	III	1980 mg L^{-1}	-22%	2015 mg L^{-1}	-20%
Cl^-	I	3210 mg L^{-1}	4%	4620 mg L^{-1}	50%
	II	2730 mg L^{-1}	-12%	2960 mg L^{-1}	-4%
	III	3520 mg L^{-1}	14%	3520 mg L^{-1}	14%
SO_4^{2-}	I	1048 mg L^{-1}	-6%	864 mg L^{-1}	-22%
	II	948 mg L^{-1}	-15%	1052 mg L^{-1}	-5%
	III	1132 mg L^{-1}	2%	1068 mg L^{-1}	-4%
Total N	I	389 mg L^{-1}	-4%	401 mg L^{-1}	-1%
	II	394 mg L^{-1}	-3%	339 mg L^{-1}	-17%
	III	348 mg L^{-1}	14%	377 mg L^{-1}	-7%
Toxicity *	I	22.68%	-35%	22.15%	-37%
	II	17.93%	-49%	23.12%	-34%
	III	11.12%	-22%	10.46%	-43%

* 25% dilution was considered for the comparison between the effluent before and after the treatments.

Besides the colour variation, Cl^-, SO_4^2, total N, and toxicity variations were monitored, too. The unmodified effluent was characterised by 2099 mg L^{-1} COD, 2550 mg L^{-1} Cl^-, 801.7 mg L^- SO_4^{2-}, 514.1 mg L^{-1} total N (Tigini et al. 2014). After the pH adjustment these values were 2532 mg L^{-1}, 3090 mg L^{-1}, 1112 mg L^{-1}, and 406 mg L^{-1}, respectively. Results about chemical and ecotoxicological analyses after biosorption treatment cycles are shown on Table 2.

The COD constantly decreased after each biosorption cycle (up to 36% after the II cycle with MUT 4050). Since the decolourisation decreased in subsequent cycles, it can be hypothesized that biomass increasingly adsorbed uncoloured organic substances, i.e. organochlorine compound, phthalates, solvents, which are often detected in landfill leachates (Jiang et al. 2013). On the contrary, humic and fulvic molecules, that are the main responsibles of leachate dark colour (Teuten et al. 2009), are probably removed in the first cycle.

A significant change in Cl^- was recorded after the first treatment cycle with biomass of *A. fumigatus* MUT 4050 (+50%). In subsequent cycles with both biomasses, Cl^- ranged from -12% to 14%.

Sulphates quite always decreased (up to 22%), in particular after the first 2 cycles. However, after each treatment cycle, the removal percentage was reduced (only 5% of removal after the III cycle). The decrease of salts was already recorded exploiting *C. berthollethiae* MUT 2861 in the biosorption treatment of textile wastewaters (Tigini et al., 2010). On the contrary, their increase can not be intuitively explained. Chlorides and SO_4^{2-} variation due to the fungal biomasses release was assessed by an additional test with a cycle of 2 hours treatment with the two biomasses put in contact with distilled water. At the end of the experiment, biomasses released a little amount of ions, since there were 26.2 mg L^{-1} Cl^- and 44.4 mg L^{-1} SO_4^{2-} with *A. fumigatus* MUT 4050, whereas there were 45.4 mg L^{-1} Cl^- and 47.8 mg L^{-1} for *C. berthollethiae* MUT 2861. However, the amount of ions released by biomasses does not justify the increase of Cl^- recorded after treatment of biosorption. Some changes in solubility of these ions may be occurred due to biosorption treatment. It must be underlined that the pH always increased up to 5.5-6 after biosorption treatment. It can be hypothesized that the hydrochloric acid, used for the pH change, caused the precipitation of NH_4Cl, which was again partially solubilised when pH increased.

The total N was subjected to weak fluctuations: after the first 2 cycles decreased up to -17% (MUT 4050), whereas after the third cycle it increased up to +14% (MUT 2861).

In order to estimate the toxicity variation with respect to the control at pH 5, the 25% dilution was considered for the comparison between samples, since this was the maximum testable dilution in common to all samples. All biomasses caused the reduction of wastewater toxicity, up to 49% reduction for MUT 2861. The toxicity reduction was not proportional to the DP and the variation of chemical parameters. Actually, the third cycle, which turned in the minimum removal of colour, salts, and ammonium, resulted in the maximum toxicity decrease for MUT 4050 (Table 2). Probably, in this case the effluent toxicity was basically due to uncoloured substances, which could be mainly removed after coloured molecules.

4 Conclusions

In this study, the best configuration for biosorption treatment with fungal biomasses was: pH 5, 5 g L^{-1} biomass cultivated on STY medium. The most effective biomasses, *Cunninghamella bertholletiae* MUT 2861 and *Aspergillus fumigatus* MUT 4050, were used in consecutive 2 h cycles in a batch biosorption experiment. The effectiveness of the treatment decreased in subsequent cycles in terms of decolourisation (31-15%). On the contrary, COD, Cl^-, SO_4^{2-}, total N, and toxicity were removed mainly in the second cycle of treatment (up to -36%, -12%, -15%, -17% and -49%, respectively). The results suggest that the effluent toxicity was basically due to uncoloured substances, which could be mainly removed after the saturation of binding sites active towards coloured molecules.

The optimised biomass of an autochthonous strain, *A. fumigatus* MUT 4050, showed a comparable sorption capacity with the biomass of the allochthonous strain, *C. bertholletiae* MUT 2861, selected and patented for its capability to adsorb dyes, salts and surfactants.

Despite the very good results, the biosorption treatment was not enough to comply the legal threshold limits about the colour and the tested ions. However, it can be considered a valid tool aimed to the improvement of the influent treatability in traditional wastewater treatment plants. In fact, the biosorption with fungal biomass potentially lead to a double advantage: the reduction of recalcitrant COD and toxicity, which could turn in the increase of the activated sludge efficiency. Moreover, the colour removal could improve the efficiency of UV disinfection processes by decreasing the absorbance of the wastewaters.

The implementation of biosorption in a recirculating stream could potentially bring to an improvement of the activated sludge efficiency. Further studies should be performed in this direction.

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