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Influence of Culture Medium on Fungal Biomass Composition and Biosorption Effectiveness

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Abstract

Zygomycetes such as *Cunninghamella elegans* seem to be promising biosorbents for pollutants removal from wastewaters because of their particular cell wall characteristics. In this article the effect of ten culture media on *C. elegans* biomass composition was investigated by means of Fourier transform infra red spectroscopy (FTIR). Biomasses grown on starches from potatoes and cereals were characterised by high amount of chitin and polysaccharides, the glucose gave rise to a biomass rich in acidic polysaccharides and lipids. By contrast, biomasses grown on corn steep liquor were poor in acidic polysaccharides and, when N sources and micronutrients were added, rich in proteins. The lipid content of the biomass generally increased by halving nutrients. Biosorption yields of these biomasses towards four wastewater models were assessed in terms of colour, salts and toxicity reduction. The biomasses rich in proteins and acid polysaccharides were less effective in removing reactive and direct dyes, whereas those rich in cationic polysaccharides showed a higher affinity for these dyes. Both chromatography and FTIR analyses showed that biomasses cultured in halved C and N had the highest affinity for salts. The wastewaters detoxification was quite always achieved, with values often lower that the Italian legal threshold limit.

Introduction

Biosorption can be regarded as an eco-friendly and cost effective alternative to traditional methods for treating textile and tannery wastewaters, which enormously contribute to water deterioration [14]. Details of adsorption mechanisms are still unclear, but surely they encompass metabolism-independent processes, which take place essentially at the cell wall level. Thus, the composition of the cell wall plays an important role in the choice of potentially exploitable organisms [6].

The fungal cell wall is a complex macromolecular structure mainly consisting of chitin and/or chitosan, glucans, mannans and proteins, but also containing other polysaccharides, lipids and pigments, e.g. melanin [4]; such a variety of structural components ensures many different functional groups, which are able to bind molecules to varying degrees [6]. This is a reason why fungal biomasses are particularly promising among different biosorbents. The fungal cell wall

composition has not been completely characterised yet and it changes in different taxonomic groups with a high intra-specific variability. Moreover, it is influenced by the fungus age, the growth medium used and the environmental conditions [4].

Zygomycetes seem to be suitable fungi for pollutants biosorption because of the composition of their cell wall, which is particularly rich (up to 50%) in acid polysaccharides such as chitin and chitosan [5, 20]. These macromolecules are characterised by amino and hydroxyl groups, which are involved in biosorption of dyes, heavy metals and phenolic compounds [2, 3].

Although it is generally known that fungi respond to the growth substrate and other physicochemical properties of the environment changing even substantially their cell wall composition, their physicochemical properties and the morphology aspect of the colony [17], few studies have analysed in detail the effects of the culture medium on the cell wall composition and, thus, on the adsorption capacity of the biomass so far.

The zygomycetes *C. elegans* was previously selected for its effectiveness in removing heavy metals, dyes and salts and characterised by means of Fourier transform infra red spectroscopy (FTIR), Differential scanning calorimetry (DSC), Thermogravimetric (TG) and contact angle analyses. It was also subjected to several physical and chemical pre-treatments in order to modify the structural features (i.e. porosity, strength, hydrophilicity) and to improve the biosorption yields [10, 19]. Besides, the lyophilised biomass of *C. elegans* was characterised in terms of adsorption isotherm and kinetics [11].

In this article we investigated, by means of FTIR analysis, the changes in biomass composition when grown on ten media characterised by different types and quantities of C and N sources. Moreover, the effect of changes in biomass composition on dyes, salts and toxicity removal yields was assessed by means of biosorption experiments towards four wastewater models designed to mime industrial effluents produced during cotton, wool and leather dyeing processes.

Materials and Methods

Simulated Wastewaters

The four simulated wastewaters were developed by the industrial partners of the EC FP6 Project SOPHIED (NMP2-CT-2004-505899). The first wastewater (W1) contained a mix of three acid dyes for wool (AY49, AR266 and Abu62; 300 ppm in total) and Na_2SO_4 (2000 ppm) at pH 5; the second wastewater (W2) contained a mix of three acid dyes for leather (ABk210, ABk194 and AY194; 300 ppm in total) at pH 5; the third wastewater (W3) contained a mix of four reactive dyes previously hydrolysed (RY145, RR195, Rbu222 and Rbk5; 5000 ppm in total) and Na_2SO_4 (70000 ppm) at pH 10; the fourth wastewater (W4) contained a mix of three direct dyes (DrY106, DrR80 and DrBu71; 3000 ppm in total) and NaCl (5000 ppm) at pH 9. All the simulated wastewaters were sterilised by tyndallization (three 1 h cycles at 60°C with 24 h interval between cycles at room temperature) before use.

Biomass Preparation

C. elegans Lendner (MUT 2861) was obtained from the Mycotheca Universitatis Taurinensis Collection (MUT, University of Turin, Plant Biology Department). The fungus, inoculated as a conidial suspension at a final concentration of 1×10^5 conidia ml^{-1}, was cultured on ten media characterised by different C and N sources, at different concentrations. They were: AM100 (18 g l^{-1}

cereals starch as C source, 2 g l^{-1} ammonium tartrate as N source, 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), ST100 (which differs from the previous medium only for the C source, 18 g l^{-1} potato starch), EQ100 (which differs from the first medium only for the C source, 20 g l^{-1} glucose), CSL_A100 (which differs from the first medium only for the C source, 20 g l^{-1} corn steep liquor), CSL_B100 (with 20 g l^{-1} Corn steep liquor as sole source of both C and N). The other five media were prepared halving C and N sources: AM50, EQ50, CSL_A50, CSL_B50. The biomasses were grown for 7 days in dynamic conditions (130 rpm) at 30°C, then they were sieved (150 μm pore) and rinsed several times with distilled water to remove residual medium, inactivated by autoclaving at 121°C for 30 min and, finally, collected in sterile conditions and rinsed as already described.

Fungal Biomasses Characterisation

All the fungal biomasses were characterised by FTIR spectroscopy. Biomass pellets were prepared in KBr discs. FTIR spectra were obtained with a Thermo Nicolet Nexus spectrometer in the 4000–400 cm^{-1} wavenumber range. Spectra were recorded by accumulating 64 scans at a resolution of 4 cm^{-1} and normalised to the 1460 cm^{-1} peak before any data processing.

Wastewater Treatment Experiments

Each biomass (0.5 g dry weight) was put in contact with 30 ml of simulated wastewaters and incubated at 30°C under agitated conditions (150 rpm). Each trial was performed in triplicate. Wastewaters without biomass were used as abiotic controls. After 2, 6 and 24 h, 200 μl of wastewaters were sampled from each flask, centrifuged at 14,000 rpm for 10 min and examined with a spectrophotometer (Amersham Bioscences Ultrospec 3300 Pro, Fairfield, CT) to acquire the complete absorbance spectra of the effluents. Since a linear relationship subsisted between the area of absorbance spectrum and the dye concentration, the dye removal percentage (DRP) was calculated as the extent of decrease of the spectrum area from 360 to 790 nm, with respect to that of the abiotic control, whose dyes concentration was known. Besides, the sorption capacity (Q_e) was calculated as follows:

$$Q_e=(V_0C_0-V_eC_e)/M$$

where Q_e is the solute uptake (mg g^{-1}); C_0 and C_e the initial and equilibrium solute concentrations in solution (mg l^{-1}), respectively; V_0 and V_e the initial and the final solution volumes (l), respectively; and M the mass of biosorbent (g).

The significance of differences ($P \leq 0.05$) among the DRP values at 2, 6 and 24 h and among Q_e values was calculated by the Mann–Whitney test (SPSS inc., 2000).

The SO_4^{2-} concentration in W3 and W1 was measured using a Dionex ICS 300 system equipped with an IonPac AS4A 4 × 250 mm column, a conductivity detector combined to a ASRS300 conductivity suppressor system (Dionex Corporation, Sunnyvale, CA, USA). The eluent was a solution of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ prepared in ultra-resi-analysed water; the flow rate was 2 ml min^{-1} and the injection volume was 25 μl. The Na^+ concentration in W3 and W1 was measured using a Dionex DX 100 system equipped with an IonPac CS12 4 × 250 mm column, a conductivity detector combined to a CSRS 300 conductivity suppressor system (Dionex Corporation, Sunnyvale, CA, USA). The eluent was a solution of 20 mM metasulfonic acid in ultra-resi-analysed water; the flow rate was 2 ml min^{-1} and the injection volume was 25 μl.

Before and after the biosorption treatment, the wastewater toxicity was measured by means of the test with the alga *Pseudokirchneriellasubcapitata* (Korshikov) Hindak (UNI EN ISO 8692:2005).

We selected this target organism as it previously proved to be the most sensitive one towards this kind of wastewaters [18].

Results

Effect of Different C and N Sources on *C. elegans* Biomass Composition

The FTIR spectra of the fungal biomasses per-cultured on different media containing 100% of C and N sources are reported in Fig. 1. The spectrum of ST100 biomass (solid line) can be taken as a reference for the description of the main spectral features attributable to the major cell wall components as already done by Tigini et al. [19].

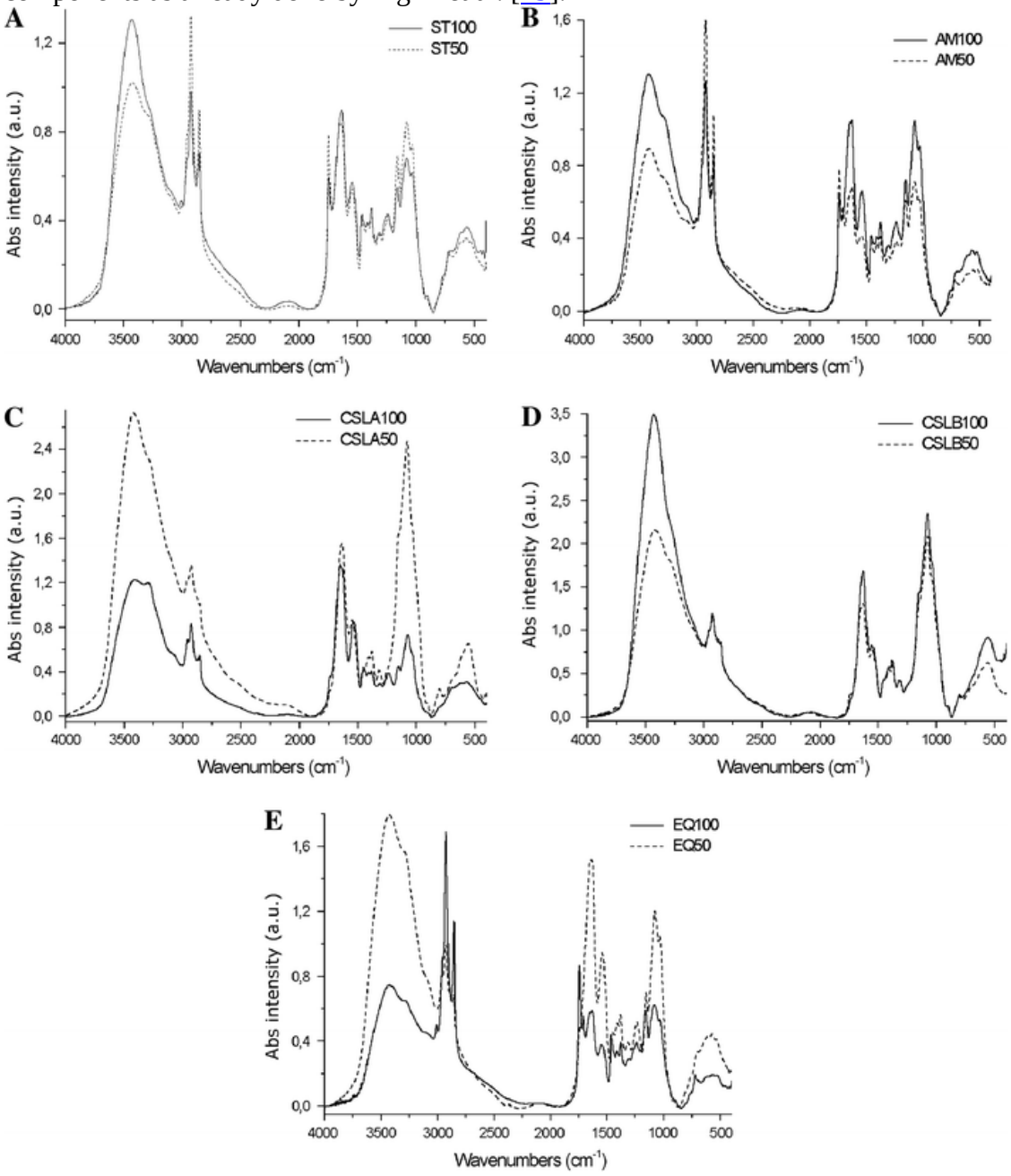

Fig. 1 FTIR spectra of *C. elegans* biomasses cultured in media containing different amounts (100 and 50%) of C and N sources: **a** ST (starch from potatoes), **b** AM (starch from cereals), **c** CSL_A (corn steep liquor), **d** CSL_B (only corn steep liquor), **e** EQ (glucose)

The biomass cultured on CSL_B100 was particularly rich in polysaccharides as confirmed by the very strong intensity of the characteristic carbohydrate backbone bands (1200–1000 cm^{-1} range), and of the corresponding O–H stretching band (3450 cm^{-1}). Acidic polysaccharides like polyglucuronic acid were only a minor component, as indicated by the low intensity of the band at about 1740 cm^{-1}. On the contrary, other acidic polysaccharides, such as chitin and chitosan were present in large amounts, as shown by the position and strength of the characteristic bands of amide I (1660–1630 cm^{-1}, C–O stretching), amide II (1570–1560 cm^{-1}, C–N stretching) and amide III (1315–1310 cm^{-1}, N–H bending). Proteins and lipids were present in relatively lower amount, as confirmed by the very weak amide III and C–H stretching bands at 2926 and 2854 cm^{-1}.

The other media resulted in more balanced biomass composition. Nevertheless, some compositional changes are worth mentioning. The biomass grown on CSL_A100, which contains corn steep liquor plus N sources and micronutrients, was poorer in acidic polysaccharides and lipids and relatively more abundant in proteins. Lipids were more abundant in biomasses grown on starch from both cereals and potatoes, ST100 and AM100 biomasses, respectively; while the biomass cultured on glucose, EQ100, displayed a remarkable higher content of lipids and acidic polysaccharides.

Effect of the Concentration of C and N Sources on *C. elegans* Biomass Composition

The biomasses cultured on ST100 and ST50 showed a very similar compositional pattern (Fig. 1a). Nevertheless, some differences came out from the FTIR spectra, when the C and N sources in the cultural medium were halved. Actually, ST50 biomass showed higher intensity of both lipids and acidic polysaccharides bands with respect to the ST100 biomass. The change in intensity in the hydroxyl groups region might be due to a different inter- and/or intra-molecular organisation of the H bonding network of the cell wall components. The growth on AM50 resulted in changes closely similar to the ones occurred in ST biomasses. Actually, this biomass had higher content of lipids and acidic polysaccharides than the AM100 one. The decrease of the amide I, II and III bands and of the polysaccharide bands is probably related to a decrease of the chitin/chitosan biomass content (Fig. 1b). With respect to CSL_A100 biomass, the CSL_A50 biomass shows a steep increase of polysaccharides content, mainly ascribable to a higher amount the chitin/chitosan components, whereas the content of acidic polysaccharides remains low and that of lipids and proteins tends to decrease (Fig. 1c). Only minor changes occurred when the C and N sources were halved in CSL_B medium (Fig. 1d). In CSL_B50 biomass the bands corresponding to hydroxyl groups decrease probably because of the lower relative moisture content of the biomass, whereas the relative amounts of lipids, proteins and polysaccharides (both acidic and chitin/chitosan) remain essentially unchanged with respect to CSL_B100. The spectrum of the biomass cultured on EQ50 showed drastic changes with respect to EQ100. Actually, lipids and acidic polysaccharides decreased sharply, while chitin/chitosan and protein components increased considerably as indicated by the stronger intensity of the amide I, II and III bands, and of the polysaccharide backbone chain vibrations (Fig. 1e).

Dyes Removal

The final dye concentration in the four wastewaters is reported in Table 1. Moreover, the wastewater DRP values are shown in Fig. 2. Substantial dye removal from W1 was nearly always achieved with DRPs from 95% (CSL_A50) to 100% (AM50). In all the cases, the process was very fast: more than 95% of the final dye removal was achieved within 2 h. The biomasses cultured on AM achieved more rapidly the final DRPs, which did not change significantly from 2 to 24 h. A total dye removal from W2 was always achieved with DRPs ranging from 99% (ST100) to 100% (CSL_B100 and CSL_B50) after 24 h. More than 99% of the final dye removal was achieved within 2 h; only CSL_B50 and CSL_A100 DRPs significantly increased after 2 h. The DRPs obtained for W3

ranged between 60 (EQ100) and 82% (AM100). From 58 to 88% of the final dye removal was achieved within 2 h. Except for CSL_B100, the DRPs always significantly increased passing from 2 to 6 h and to 24 h. The DRPs obtained for W4 ranged between 80 (EQ100) and 100% (CSL_B100). The process of dye removal from W4 was the slower one; actually, from 39 to 74% of the final dye removal was achieved within 2 h. The DRPs always significantly increased passing from 2 to 6 h and to 24 h.

Table 1

Dye concentration at the end of the biosorption treatment of W1, W2, W3 and W4 effluents

	Final dye concentration (mg l^{-1})			
	W1	**W2**	**W3**	**W4**
AS100	2	0	882	149
AS50	2	2	1094	231
AF100	6	2	900	23
AF50	0	0	979	108
EQ100	5	1	2017	609
EQ50	3	1	1217	451
CSL100	6	0	1089	0
CSL50	13	0	1830	387
CSL-EQ100	10	1	1175	276
CSL-EQ50	15	0	1795	210

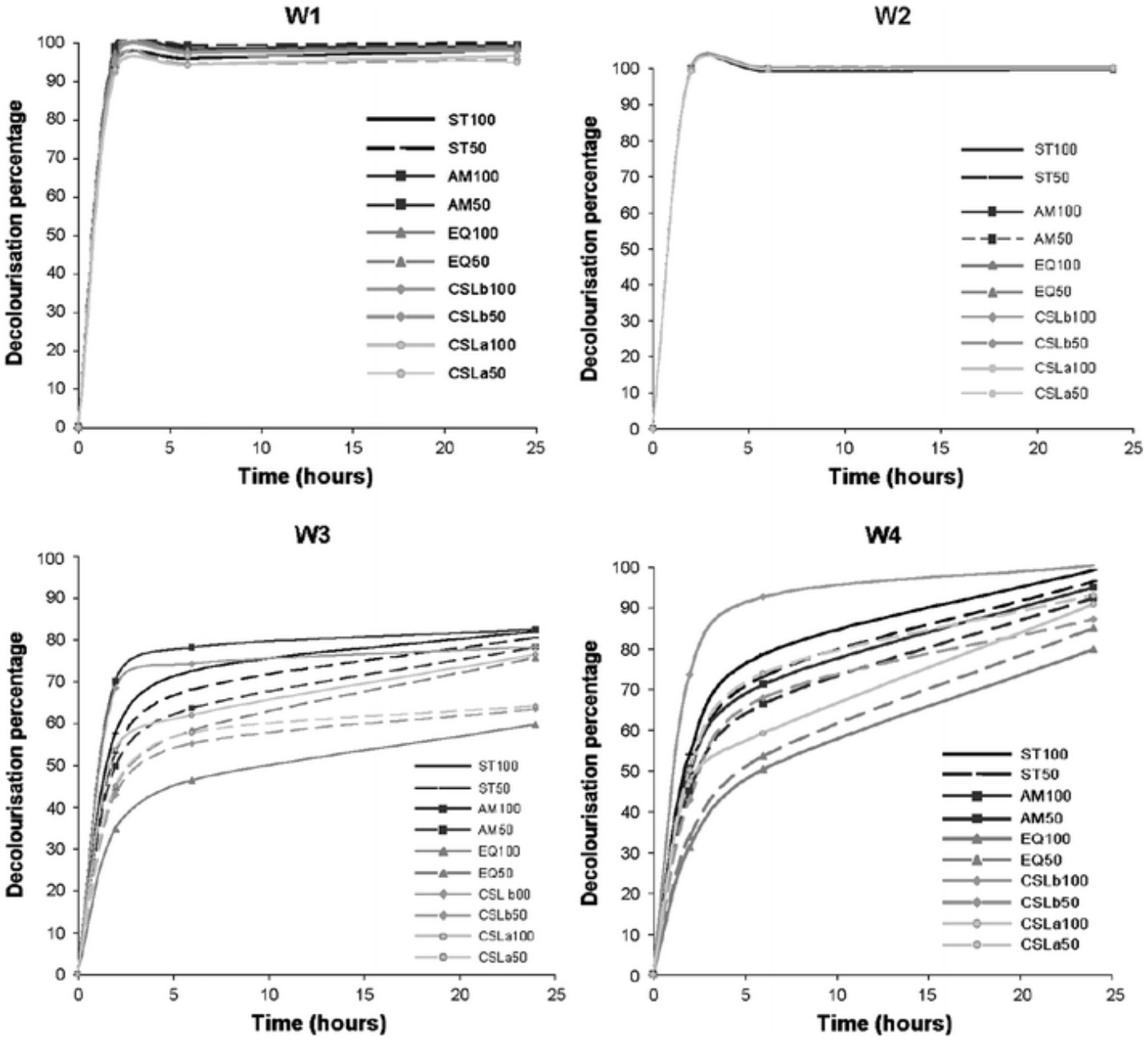

Fig. 2

Dye removal percentage from W1, W2, W3 and W4 after 24 h incubation by the different biomasses

The Q_e values of the fungal biomasses towards the W1, W2, W3 and W4 effluents are listed in Table 2. The different biomasses quite always showed similar Q_e towards W1 and W2: their values ranged from 17 mg g^{-1} (CSL_A50 towards W1) to 18 mg g^{-1} (ST100 towards W1). The effect of medium composition on biomass Q_e was particularly evident in the case of wastewaters with high dye concentration (W3 and W4). The highest Q_e were displayed towards W3 by all the fungal biomasses, with values ranging from 179 mg g^{-1} (EQ100) to 251 mg g^{-1} (ST100). Whereas, towards W4, biomasses showed Q_e ranging from 144 mg g^{-1} (EQ100) to 183 mg g^{-1} (ST100). The biomasses cultured in starches and in different CSL displayed the highest Q_e values towards reactive and direct dyes. Quite all the biomasses cultured in media with halved C and N sources displayed significantly lower Q_e values with respect to the correspondent biomasses cultured on media with 100% C and N, with the exception of biomasses cultured on EQ.

Table 2

Sorption capacity Q_e (mg of dye g^{-1} of biomass dry weight) of inactivated biomasses cultured on different media towards W1, W2, W3 and W4 effluents

Biomass	Q_e (mg of dye g^{-1} of biomass dry weight)			
	W1	W2	W3	W4
AM100	17.9 ± 0.1^{aA}	18.0 ± 0.0^{aB}	247.1 ± 1.8^{aC}	171.1 ± 9.3^{aD}
AM50	17.9 ± 0.1^{abA}	17.9 ± 0.1^{bA}	234.4 ± 4.1^{bC}	166.1 ± 1.3^{bD}
ST100	18.0 ± 0.1^{bcdA}	18.3 ± 0.0^{bB}	251.4 ± 7.8^{aC}	182.7 ± 0.2^{bcD}
ST50	18.0 ± 0.1^{aceA}	18.0 ± 0.0^{bA}	241.3 ± 2.5^{bdC}	166.1 ± 1.3^{bD}
EQ100	17.7 ± 0.1^{gfdA}	18.0 ± 0.0^{bB}	179.0 ± 7.0^{cC}	143.5 ± 0.6^{dD}
EQ50	17.8 ± 0.0^{befA}	18.0 ± 0.0^{bB}	227.0 ± 7.7^{bdC}	152.9 ± 3.0^{eD}
CSLB100	17.6 ± 0.1^{agA}	18.0 ± 0.0^{cB}	234.7 ± 3.5^{dC}	180.5 ± 0.6^{fD}
CSLB50	17.2 ± 0.1^{hiA}	18.0 ± 0.0^{bcB}	190.2 ± 2.6^{eC}	156.8 ± 3.1^{eD}
CSLA100	17.4 ± 0.1^{hA}	18.0 ± 0.0^{Bb}	229.5 ± 5.0^{bdC}	163.4 ± 0.0^{cD}
CSLA50	17.0 ± 0.2^{iA}	18.0 ± 0.0^{bcB}	192.3 ± 6.4^{eC}	169.9 ± 4.7^{bD}

Small letters indicate significant differences between the Q_e of different biomasses towards the same effluent. *Capital letters* indicate significant differences between the Q_e of the same biomass towards different effluents

Salts Removal

In W1, the Na^+ and SO_4^{2-} concentrations were 790 and 1405 mg l^{-1}, respectively. The highest Na^+ removal percentage (32%) was achieved by AM50 biomass, whereas the highest SO_4^{2-} removal percentage (32%) was achieved by ST50 biomass (Fig. 3). In W3, the initial Na^+ and SO_4^{2-} concentrations were 30.7 and 51.5 g l^{-1}, respectively. The AM50 biomass showed the highest removal percentages for both Na^+ and SO_4^{2-}, with a decrease of 38 and 57%, respectively (Fig. 3).

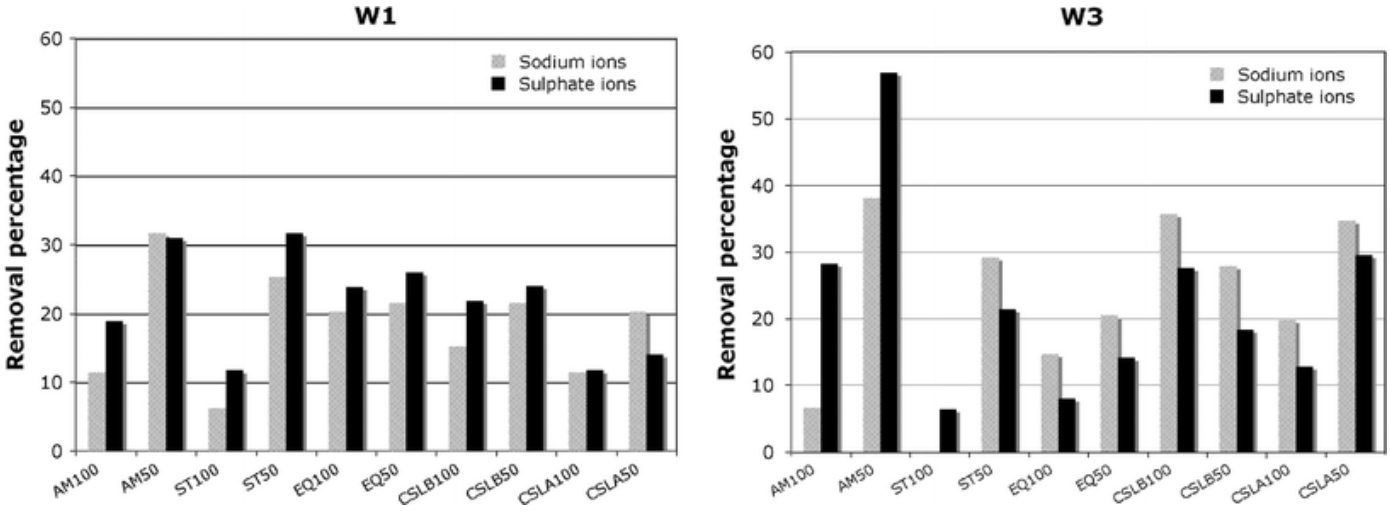

Fig. 3 Na^+ and SO_4^{2-} removal percentage in W1 and W3 after the treatment with biomasses cultured on different media

Biomasses Characterisation after Biosorption Treatment of Wastewaters

The FTIR spectra indicate that two strong peaks located at 1200–1000 cm^{-1} and at 650–600 cm^{-1} were always found in all the spectra of the biomasses put in contact with W3 (Fig. 4). These two peaks can be attributed to the characteristic absorptions of the inorganic SO_4^{2-}. In particular, the strong bands appearing at about 1200–1050 cm^{-1} are due to out of phase asymmetric stretching, the weaker components at about 1000 cm^{-1} are due to symmetric in phase stretching, and the sharp bands at 650–600 cm^{-1} are assigned to the bending mode of SO_4^{2-}.

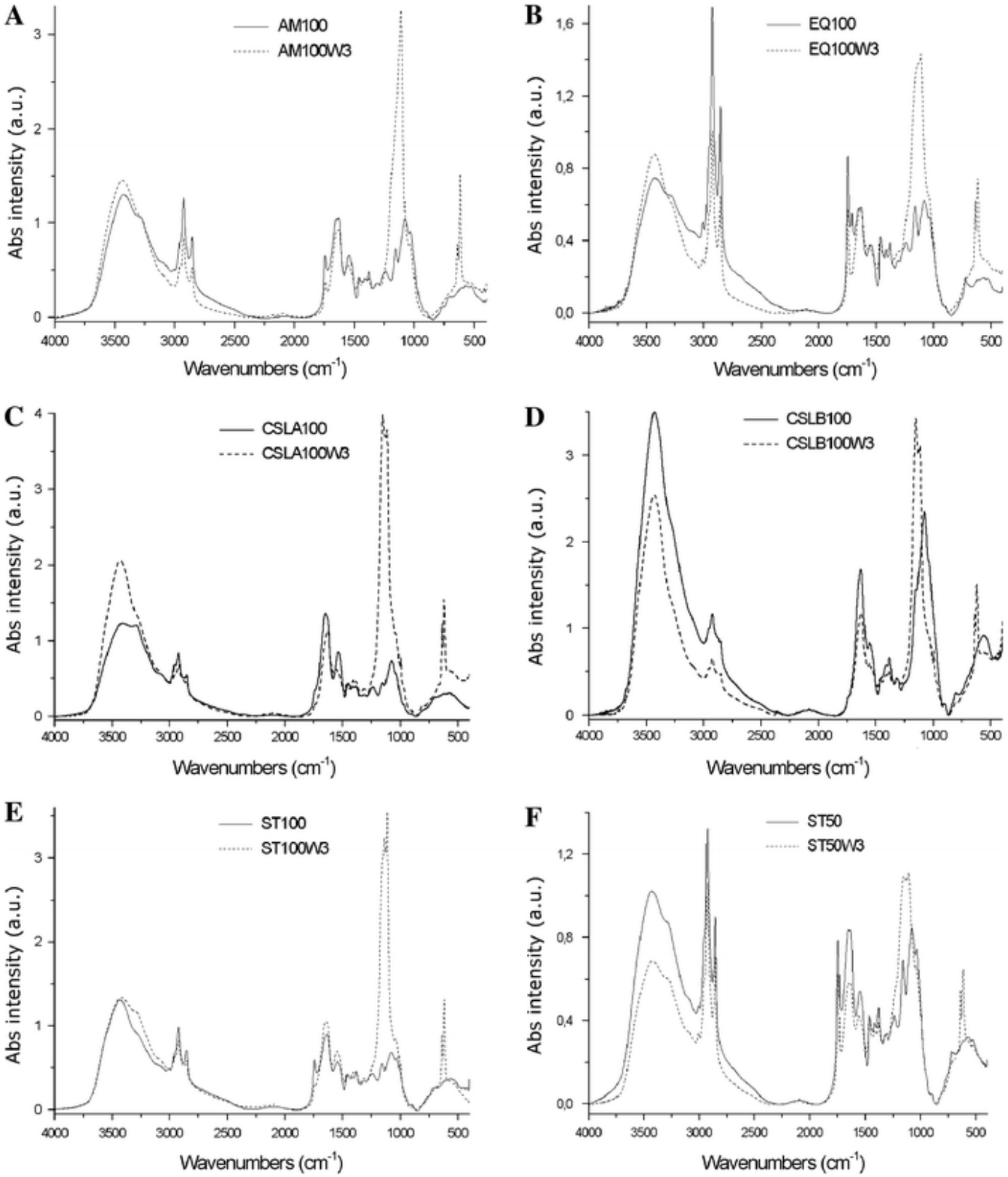

Fig. 4 FTIR spectra of biomasses before and after the treatment with W3. **a** AM100 (starch from cereals); **b** EQ100 (glucose); **c** CSL_A100 (corn steep liquor); **d** CSL_B100 (only corn steep liquor); **e** ST100 (starch from potatoes); **f** ST50 (halved amount of starch from potatoes)

An interesting feature appearing from the FTIR spectra is that the shape and relative intensity of SO_4^{2-} stretching and bending bands change in the different biomasses. As an example, the 1300–550 cm^{-1} spectral range of samples ST100, CSL_A100 and AM100 is reported in Fig. 5.

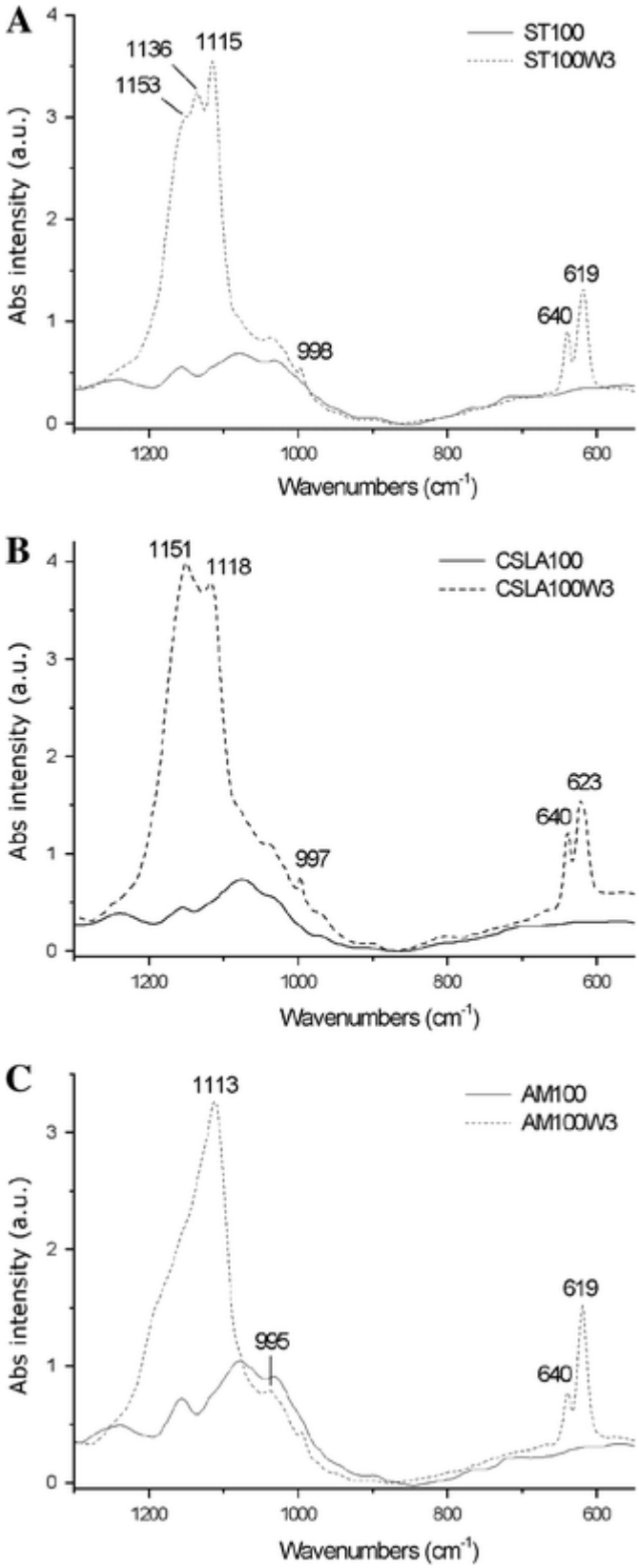

Fig. 5 FTIR spectra in the 1300–550 cm^{-1} range of biomasses ST100 (**a**), CSL_A100 (**b**), AM100 (**c**) before and after the treatment with W3

Finally, some changes in the biomass spectra were observed in the vibration modes related to hydroxyl and amino groups, hydrocarbons, amide I, amide II, amide III and ether groups (1157–1032 cm^{-1}), which are overall attributable to a complex range of interactions occurring between the

effluent components (mainly salts and dyes) and the biomass polymers (polysaccharides, proteins and lipids).

Ecotoxicity Test

Since the data distribution of the treated samples resulted in a very flat sigmoid dose-effect chart the EC_{50} was not always calculable. Thus, in order to evaluate the reduction of the toxicity after biosorption, the results were elaborated comparing the effect caused by the highest tested dose of the treated sample to that of the untreated one. For W1, W2 and W4, the toxicity reduction percentages were calculated comparing the 25% wastewater dilution dose; whereas, for W3 the selected dilution dose was 3% because of its high toxicity. The results are reported in Table 3. After the biosorption treatment with fungal biomasses, the wastewaters toxicity decreased in quite all cases, with decrease percentages ranging from 11 to 100%. The best results towards W1 and W4 were achieved by AM100, ST50 determined the highest detoxification rate towards W2 and EQ50 towards W3.

Table 3

Toxicity reduction percentage of W1, W2, W3 and W4 effluents after the biosorption treatment with inactivated biomasses cultured on different media

Biomass	W1 (25% dose)	W2 (25% dose)	W3 (3% dose)	W4 (25% dose)
AM100	99.1*	77.6*	48.9*	96.9*
AM50	76.3*	79.3*	52.7*	81.8*
ST100	93.8*	11.0*	55.0*	90.8*
ST50	86.0*	100.0*	64.9*	78.0*
EQ100	74.6*	91.1*	0.0	37.9*
EQ50	65.8*	86.7*	76.6*	14.0*
CSLB100	20.8*	74.3*	35.3*	0.0
CSLB50	47.8*	48.4*	18.9*	23.1*
CSLA100	63.7*	47.8*	12.5*	2.0
CSLA50	52.8*	74.7*	37.3*	26.7*

* Indicates significant differences ($P < 0.05$, Mann–Whitney test) with respect of untreated effluent

Discussion

While influence of abiotic parameters on biosorption yields has been extensively studied so far [12], few data are currently available on biotic parameters. The results of this study indicate that the origin of C and N sources causes significant changes in the composition of the fungal cell wall. In particular, biomasses grown on starches, both from cereals and potatoes, were characterised by the presence of all the main cell wall components, with high amount of chitin and polysaccharides too. Starch from yam bean was shown to be a good C source for producing *C. elegans* biomass rich in chitin and chitosan [15]. The use of glucose resulted in a biomass rich in acidic polysaccharides and lipids. By contrast, the use of corn steep liquor resulted in biomasses very poor in acidic polysaccharides. The addition of N sources and micronutrients to corn steep liquor medium resulted in a biomass very rich in proteins with respect to the one cultured on the sole corn steep liquor. This substrate consists of a complex mixture of substances deriving from the wet milling process of maize-starch industry and it has been reported to be a good source of protein for fungi, such as

Aspergillus parasiticus and *A. niger* [8, 13]. Nevertheless, from the present study it seems that *C. elegans* need the addition of simpler N source to produce cell wall proteins.

Also the concentration of C and N sources determined changes in the cell wall composition; generally, in fact, the lipid content of the biomass increased by halving the nutrients. These changes in cell wall composition turned into different dye removal yields. This effect was evident in particular towards the most concentrated wastewaters. The biomasses rich in protein were less effective in removing reactive and direct dyes. On the contrary, biomass rich in cationic polysaccharides, such as chitin or chitosan, showed high affinity for these dyes. Reactive and direct dyes, in fact, are formulated for dyeing cellulose fibres and their affinity for acidic polysaccharides is well-documented in the literature [2, 3, 14].

From an applicative point of view, the AM and CSL media are very promising due to their low cost at laboratory scale (about 0.5 € l^{-1}) with respect to the other ones (that exceed 0.9 € l^{-1}). This two media could be competitive also with respect to by-product fungal biomasses extensively used in industrial fermentation processes. Actually, even if the use of waste-biomasses in biosorption application could be helpful in solving solid waste disposal problems in a cost-friendly way [3], till now the potential use of fungal waste-biomasses in the removal of pollutants remains largely untapped and almost exclusively limited to heavy metals [6, 7, 16, 21].

FTIR analyses showed that all the biomasses adsorbed SO_4^{2-} from W1 and W3 even if some distortions of the ion peak complicated the spectra interpretation. A distortion of symmetry, for example by electrostatic effect, may shift the stretching peak of SO_4^{2-} at about 1100 cm^{-1} to higher wavenumbers and cause the appearance of a lower wavenumber component at about 1000 cm^{-1}, i.e. a symmetric stretch that is usually forbidden when the environment around SO_4^{2-} is also symmetrical. If the degree of symmetry of the SO_4^{2-} is further decreased, for example when it forms bridging complexes in form of film on the surface of other materials, or when it interacts in the solid state with other materials, such as with the polymers comprising the biomasses, the main stretching band is split in at least three components falling between 1200 and 1050 cm^{-1}, and the bending band is also split into more components. Therefore, different shape and relative intensity of SO_4^{2-} stretching and bending modes observed in the FTIR spectra of biomasses after contact with W3 may reflect different absorption and binding states of the SO_4^{2-}, which in turn may depend on the different composition and texture of the fungal cell wall. The results reported in this study makes it difficult to establish a clear relationship between specific biomass compositional features and the strength and mode of binding, and the mechanism underlying the interactions of SO_4^{2-} with the biomass polymeric components. Indeed, this is a topic worth of further investigations.

Chromatography analyses showed that biomasses cultured in media with halved C and N displayed the highest affinity for salts. A possible explanation for this result could be the involvement in salts binding of CH groups of lipids carbon chains, which are particularly abundant in these biomasses. Actually, lipids, in particular phospholipids, are reported as binding site for heavy metals [9], but they have never been pointed out for salt biosorption up to now. From an applicative point of view these good results are very important, since about 75% of the huge amount of salts used during dyeing processes is released in water stream because of the inefficacy of traditional wastewater treatment plants [1]. Hypersaline wastewaters can seriously damage the aquatic ecosystem and can compromise the fertility of soil by the irrigation with polluted water; moreover they can also damage the underground fresh water reserves by soil percolation. Despite international awareness towards the protection of these resources, only 15% of publications consider this aspect in the qualitative assessment of wastewaters [1].

After biosorption treatment, the wastewaters toxicity often decreased under the legal threshold value fixed by the Italian law (DM 152/2006). Only W3, which has the highest concentration of dyes and salt, still showed high toxicity after biosorption treatment. The biomass effectiveness in detoxification was not always proportional to the removal of both dye and salt. This was particularly evident in the case of W1 and W2, towards which biomasses showed significantly different detoxification yields, despite very similar dye removal percentages. Probably, biomasses differently adsorbed other auxiliaries present in dye powders, such as surfactants, not detected in this experiment. Actually, dye powder purity is guarantee from 30 to 90%.

In conclusion, these experiments point out some important aspects of the chemical composition of *C. elegans* biomass and its changes due to culture media. Moreover, a systematic approach has been undertaken in this study to address key aspects of the biosorption process, such as the affinity according to which dyes and other molecules are adsorbed and the role of the functional groups involved.

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