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Cunninghamella elegans biomass optimisation for textile wastewater biosorption treatment: an analytical and ecotoxicological approach

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Abstract

The effect of pre-treatments on the composition of *Cunninghamella elegans* biomass and on its biosorption yields in the treatment of simulated textile wastewaters was investigated. The inactivated biomass was subjected to physical treatments, such as oven drying and lyophilisation, and chemical treatments using acid or alkali. The waste- water colour, COD and toxicity variations were evaluated. The lyophilisation sped up the biosorption process, whereas the chemical pre-treatment changed the affinity of biomass for different dyes. The alkali per-treated biomass achieved the highest COD reduction in the treatment of alkali wastewaters, probably because no release of alkali-soluble biomass components occurred under the alkaline pH conditions. Accordingly, only the acid pre-treated biomass decreased the COD of the acidic effluent. The ecotoxicity test showed significant toxicity reduction after biosorption treatments, indicating that decolourisation corresponds to an actual detoxification of the treated wastewaters. Fourier transform infrared spectroscopy, differential scanning calorimetry and thermogravimetric analyses of biomasses allowed highlighting their main chemical and physical properties and the changes induced by the different pre- treatments, as well as the effect of the chemical species adsorbed from wastewaters.

Introduction

The textile industry generates huge amounts of coloured wastewaters, which contribute enormously to water deteri- oration (Banat et al. 1996; Vijayaraghavan et al. 2008). Unfortunately, conventional treatment techniques are not always effective towards textile effluents that are one of the most difficult-to-treat wastewaters on account of their considerable amount of suspended solids, high chemical demand and the massive presence of weakly biodegradable and often toxic substances. Therefore, efficient, eco- friendly and cost-effective remedies for wastewater treat- ment are needed (Vijayaraghavan et al. 2008).

In the last decades, several researches have shown that biosorption can be regarded as a valid alternative to traditional methods and to microbial or enzymatic biodeg- radation (Vijayaraghavan et al. 2008). The main attractions of biosorption are high efficiency, cost effectiveness and good removal from large volumes (Aksu 2005; Gadd 2009). Among the different biosorbents tested against dyes so far, dead fungal biomass has proved to be particularly suitable, presenting several advantages among the living ones: they are not affected by toxic wastes, do not require nutrients and do not release toxins or propagules (Crini 2006; Prigione et al. 2008a; Anastasi et al. 2009).

Despite the established benefits of biosorption and the huge amount of publications on this topic, applications at industrial level are virtually absent probably because of the still low robustness of biomass-based systems. Thus, some authors suggested that the attention should be focused on biomass modifications, alteration of bioreactors configuration and physico-chemical conditions to enhance biosorption (Gadd 2009).

Moreover, recent literature indicates the need to generate performance data on real or simulated industrial effluents since many biotic and abiotic factors can affect the biosorption process (Aksu 2005; Gadd 2009; Kaushik and Malik 2009). Nevertheless, most of the studies on bio- sorption focus on dye removal from single dye solutions and only few with multicomponent solutions have been carried out so far (Khalaf 2008; Prigione et al. 2008b).

Another aspect that must be considered is the effluent toxicity and its evolution during wastewater treatment, as required by the Integrated Pollution Prevention and Control regulations introduced in the European Union. In some cases, in fact, the decolourisation results in the formation of colourless but toxic and mutagenic compounds provoking an increase of the wastewater toxicity (Pearce et al. 2003; Keenan et al. 2007; Sharma et al. 2007).

The zygomycetes *Cunninghamella elegans* was previously selected among many fungal strains for its effectiveness in removing single and mixed dyes (Casieri et al. 2008; Prigione et al. 2008a, b). In order to optimise the biosorption yields for industrial application, this paper investigates the effect of physical and chemical pre-treatments of *C. elegans* biomass in biosorption experiments, towards wastewater models designed to mime wastes produced during cotton and wool dyeing processes. Moreover, the COD measurements were performed before and after the treatment, and changes in effluent toxicity were estimated by the *Pseudokirchneriella* subcapitata ecotoxicity test. Finally, Fourier transform infra- red spectroscopy (FTIR) analysis, differential scanning calorimetry (DSC) and thermogravimetric analysis (TG) were used to characterise the native and pre-treated biomasses before and after biosorption.

Experimental section

Simulated wastewaters

Three simulated wastewaters, developed by the industrial partners of the EC FP6 Project SOPHIED (NMP2-CT-2004-505899), were designed to mime effluents produced during cotton or wool textile dyeing processes and were used under the permission of the SOPHIED Consortium. The first wastewater (W1) contained a mix of three acid dyes (AY49, AR266 and Abu62; 300 ppm in total) and Na_2SO_4 (2,000 ppm); it had an ionic strength of 4.2×10^{-2} and pH 5. The second wastewater (W2) contained a mix of four reactive dyes previously hydrolysed (RY145, RR195, Rbu222 and Rbk5; 5,000 ppm in total) and Na_2SO_4 (70,000 ppm); it had an ionic strength of 1.48 and pH 10. The third wastewater (W3) contained a mix of three direct dyes (DrY106, DrR80, and DrBu71; 3,000 ppm in total) and NaCl (5,000 ppm); it had an ionic strength of 8.6×10^{-2} and pH 9. All the simulated wastewaters were sterilised by tindalisation (three 1-h cycles at 60 °C with 24-h interval between cycles at room temperature) before use.

Test organisms and fungal biomass preparation

Cunninghamella elegans Lendner (MUT 2861) was obtained from the *Mycotheca Universitatis Taurinensis* Collection (MUT, University of Turin, Department of Plant Biology). The fungus was cultured on ST, a medium rich in starch, and heat-inactivated as already described (Prigione et al. 2008a).

A part of the biomass was chemically pre-treated, putting it in 0.1 M HCl solution for 4 h at room

temperature under agitated conditions or boiled in 0.5 M NaOH solution for 15 min. Then, biomasses were washed with deionised sterilised water as long as the pH of the washing solution was in the near-neutral range (7.0–7.2). Another part of C. *elegans* biomass was dried in an oven at 65 °C for 24 h, or lyophilised (Lyophiliser LIO 10P; Cinquepascal, Trezzano s/n, Italy), then powdered and sieved into four different size ranges: 1,000–600 μm, 600–300 μm, 300–150 μm and ≤150 μm.

Fungal biomasses characterization

All the 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 4,000–400 cm^{-1} wavenumber range. Spectra were recorded by accumulating 64 scans at a resolution of 4 cm^{-1} and normalised to the 1,460 cm^{-1} peak before any data processing.

The thermal behaviour of each biomass was studied by means of DSC and TG analyses. DSC was performed using a Mettler DSC 30 instrument, calibrat- ed with an indium standard. The calorimeter cell was flushed with 200 ml min^{-1} of N_2. Sample weight was about 3 mg and Al crucibles were used. The temperature range scanned was 25–500 °C at a heating rate of 10 °C min^{-1}. TG was performed with a TGA Q500 instrument (TA Instruments) from room temperature to 1,000 °C on 3-mg samples. The cell was swept with N_2 during the analysis.

Biosorption experiments

Each biomass was weighed and 0.5 g of dry weight was placed in 50-ml Erlenmeyer flasks containing 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 was sampled from each flask, centrifuged at 14,000 rpm for 10 min and examined with a spectrophotometer (Ultrospec 3300 Pro; Amersham Biosciences, Fairfield, CT, USA) to acquire the complete absorbance spectra of the effluents. Since a linear relationship subsisted between the area of absorbance spectrum and dye concentration, the percentage of removed dye (DP, decolourisation percentage) was calculated as the extent of decrease of the spectrum area from 360 nm to 790 nm, with respect to that of the abiotic control, whose dye concentration was known.

Besides, the sorption capacity (Q_e) was calculated as follows:

Q_e = mg of removed dye=g of biomass dry weight (1)

The significance of differences ($P \leq 0.05$) among the DP values at 2, 6 and 24 h and among Q_e values was calculated with the Mann–Whitney test (SPSS Inc. 2000). At the end of the experiment, wastewaters were filtered (Whatman type 1 filter paper) for COD and ecotoxicity measurement, and the exhausted biomasses were characterised as previously described.

COD measurement and ecotoxicity test

Determination of COD with the dichromate method was performed using HACH (COD high-range vials) apparatus according to the manufacturer's instructions. A calibration curve was obtained using HACH COD standard solution (800 mg O_2 l^{-1}). Appropriate dilutions of each sample were assayed.

Wastewater toxicity was measured before and after the biosorption treatments by means of the alga *Pseudokirchneriella subcapitata* (Korshikov) Hindak (UNI EN ISO 8692:2005). Each dose–response curve consisted of 12 dilutions, each one in three replicates. Controls were performed in six replicates. The final algal cell density

was determined by electronic particle counting using a Coulter counter (Beckman).

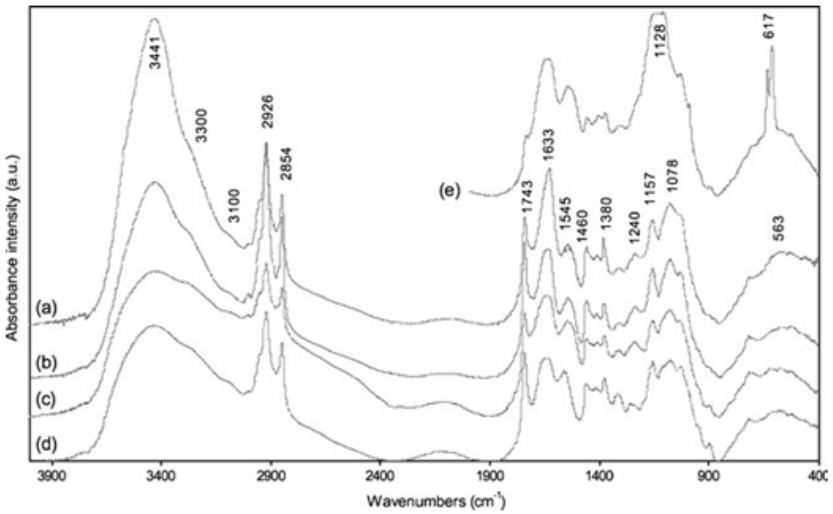
Results

Biomasses characterisation before the biosorption experiments

The biomass FTIR spectra are shown in Fig. 1. The spectrum of the lyophilised biomass (Fig. 1a), which can be taken as a reference for the description of the main spectral features, is characterised by a strong and broad absorption at 3,441 cm^{-1} attributed to stretching vibrations of O–H groups of polysaccharides and proteins involved in H bonding. The two shoulder peaks falling at about 3,300 cm^{-1} and 3,100 cm^{-1} represent N–H stretching vibrations of the amide group of proteins and of the acetamido group of the polysaccharide chitin, one of the main components of zygomycetes cell wall. The sharp peaks at 2,926 cm^{-1} and 2,854 cm^{-1} are assigned to C–H stretching of hydrocarbon chains of lipids, while the absorption band at 1,743 cm^{-1} represents the C=O stretching vibration of unionised carboxylic groups of acidic polysaccharides like polyglucuronic acids. The amide vibrations of the peptide bond of proteins and of the acetamido group of chitin overlap in the spectrum of the lyophilised biomass. Characteristic peaks appear at about 1,633 cm^{-1}, 1,545 cm^{-1} and 1,240 cm^{-1} for amide I, II and III, respectively. An amide III component of chitin can also be observed at 1,309 cm^{-1}. Various weak-to- medium intensity methyl and methylene bending vibra- tions of hydrocarbon chains fall between 1,460 cm^{-1} and 1,380 cm^{-1}. Polysaccharide backbone chains give rise to strong absorption bands in the 1,200–1,000 cm^{-1} range, which can be assigned to various vibrations of different carbohydrate moieties (C–O–C ring stretching and C–O stretching of primary and secondary alcohols).

A common feature of the FTIR spectra of oven-dried (Fig. 1b), acid- (Fig. 1c) or alkali-treated (Fig. 1d) biomasses is the change in shape and relative intensity of the bands falling in the O–H/N–H stretching range at above 3,000 cm^{-1}. Other spectral changes are more peculiar of one specific treatment. For example, oven drying caused a marked intensity increase of the C–H stretching bands at about 3,000–2,800 cm^{-1} and of the carbonyl stretching band at 1,743 cm^{-1}. The carbonyl stretching band remained almost unchanged upon treatment with acid or alkali. On the other hand, both acid and alkali resulted in a lower intensity of the carbohydrate bending vibrations at about 1,100–1,000 cm^{-1}. Interestingly, the alkali pre-treated biomass displayed drastic changes in the shape and intensity of the amide bands, which shifted from the mixed chitin/protein pattern characteristic of the lyophilised biomass to the chitin-like pattern of the alkali-treated biomass. This effect is probably due to loss of protein material. Resuming, the physical pre-treatments did not change the chemical composition of the biomass; on the contrary, the chemical pre-treatments strongly affected the biomass composition: HCl pre-treatment decreased the chitosan/chitin biomass content (amide groups), while the NaOH decreased the protein/glycoprotein biomass content.

Fig. 1 FTIR spectra of the different fungal biomasses: a lyophilised; b oven dried; c treated with acid; d treated with alkali. The spectrum (e) shows the FTIR profile in the 2,000–400 cm^{-1} range of the lyophilised biomass analysed after exposition to effluent W2



The thermal behaviour of the physically and chemically pre-treated biomasses was examined by DSC, and thermo- grams are shown in Fig. 2. The lyophilised biomass (Fig. 2a) is characterised by a broad endotherm peaking at around 80 °C, which is attributed to the evaporation of moisture. This intense transition is likely to overlap other low-temperature thermal transitions attributable to lower molecular weight and/or less thermally stable biomass components such as lipids.

The broad endothermic events falling at higher temperature are rather difficult to attribute to individual molecular species. Those falling at 200–300 °C can be related to thermal transitions of protein and polysaccharide macromolecules, which are known to present melting events and onset of thermal degradation in this range. The transitions falling at higher temperature can be attributed to biomass components or phases with a more compact and probably highly cross- linked texture, which display enhanced thermal stability.

The thermal behaviour of the biomass showed significant changes upon drying (Fig. 2b). The moisture evaporation endotherm broadened significantly with a peak at above 100 °C. Accordingly, high-temperature endotherms shifted upwards, indicating that oven drying strongly changed the chemical and physical texture of the biomass. The biomass treated with acid behaved closely similar to the lyophilised one (Fig. 2c), while the main DSC feature of the biomass treated with alkali is the absence of the protein-related endothermic transition at about 235 °C (Fig. 2d). This last finding is in agreement with FTIR results, which showed that alkali preferentially remove the biomass protein fraction.

The kinetics of weight loss during heating of fungal biomasses is shown in Fig. 3. The trend of the TG curves is broadly similar for all biomasses, with the onset of extensive weight loss located at about 220–230 °C, slightly higher for the biomass treated with alkali due to the loss of the thermally sensitive protein fraction. Afterwards, the

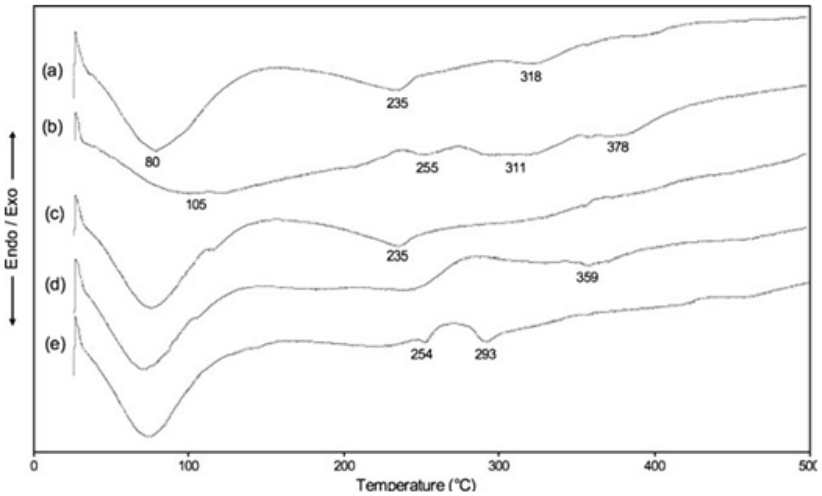


Fig. 2 DSC thermograms of the different fungal biomasses: a lyophilised; b oven dried; c treated with acid; d treated with alkali. The thermogram (e) shows the DSC profile of the lyophilised biomass analysed after exposition to W2

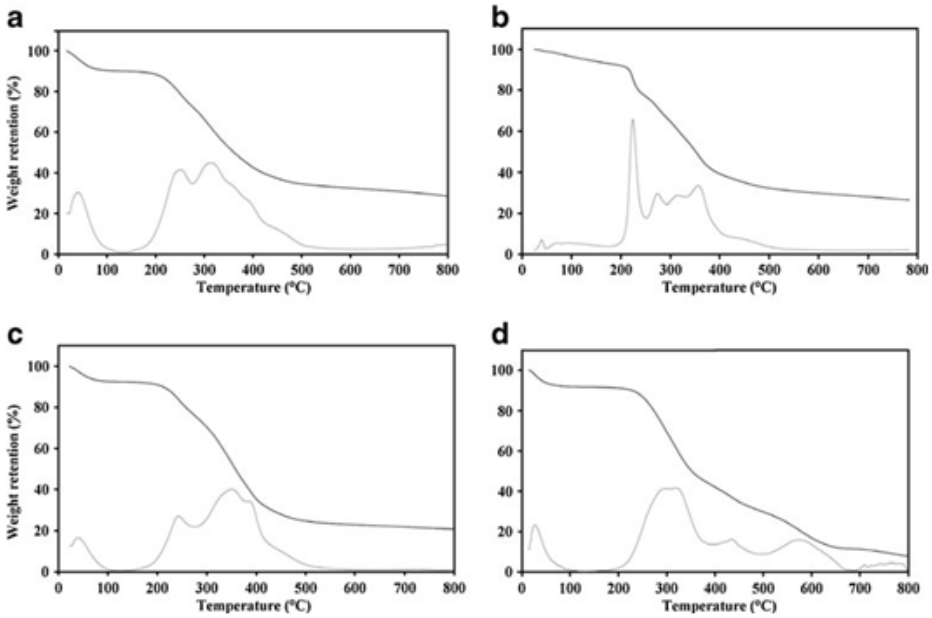


Fig. 3 TG curves of the different fungal biomasses. Black line, weight retention curve; grey line, derivative curve. a Lyophilised biomass. b Oven-dried biomass. c Biomass treated with acid. d Biomass treated with alkali

weight tends to a plateau at above 500 °C. It is interesting to note that the oven-dried biomass showed a different trend of the initial weigh loss below 100 °C (moisture evaporation) compared to the other biomasses. This behaviour is in agreement with DSC results, suggesting a stronger binding strength of water mole- cules with biomass components and a change in the overall biomass texture upon drying. The values of weight retention at 800 °C are 28.4%, 26.5%, 20.7% and 7.8% for lyophilised, oven-dried, acid- and alkali-treated biomasses, respectively.

Alkali significantly decreased the weight retention at high temperature, meaning that biomass fractions formerly stable became more temperature sensitive upon treatment under alkaline conditions. Acid was less effective in reducing the thermal stability of the biomass, while no differences can be observed in this respect between lyophilised and oven-dried biomasses.

Chemically pre-treated biomass effectiveness

The DP values of W1, W2 and W3 effluents achieved by the untreated biomass and chemically pre-treated bio- masses and of *C. elegans* are shown in Fig. 4. In all cases, more than 94% of the final decolourisation was achieved within 2 h. At the end of the experiment, DP of W1 achieved by chemically

pre-treated biomasses ranged between 61% and 98%. The acid pre-treatment did not affect the decolourisation process of W1 with respect of the untreated biomass, whereas the alkali pre-treatment significantly decreased (−61%) the decolourisation yields within 2 h. Considering the DPs for W2 obtained within 2 h, the alkali pre-treated biomass achieved DP similar to the untreated biomass and was 31% higher than the acid one. At the end of the experiment, the DPs achieved by chemically pre-treated biomasses were very similar and ranged between 76% and 78% resulting less effective than the untreated one (DP 82%).

Alkali pre-treatment sped up the biosorption process (+40%) towards W3 with respect to the untreated biomass, whereas the acid pre-treatment slowed down the process (−12%). Nevertheless, at 24 h, chemically pre-treated biomasses achieved similar DP (97–98%) and were slightly, but significantly, less effective than the untreated biomass (DP 99%).

Chemical pre-treatments quite always resulted in little but significant reduction biomass sorption capacity (Table 1) .

Chemically pre-treated biomasses almost always produced a decrease of COD values: only alkali pre-treated biomass caused an increase of COD in W1. The biomass pre-treated with HCl always caused a higher COD reduction than the untreated one, whereas the biomass pre-treated with NaOH showed different trends according to the wastewater (Table 2).

The results of ecotoxicity test were elaborated comparing the effect caused by the highest tested dose of the treated sample to the corresponding one of the untreated wastewater (Table 2). The highest toxicity reduction of W1 was achieved by the untreated biomass, followed by the acid and alkali pre-treated ones. On the contrary, towards W2, the alkali pre-treated biomasses achieved the highest detoxification yield followed by the acid and the untreated biomasses. Towards W3, the highest toxicity reduction was achieved by acid pre-

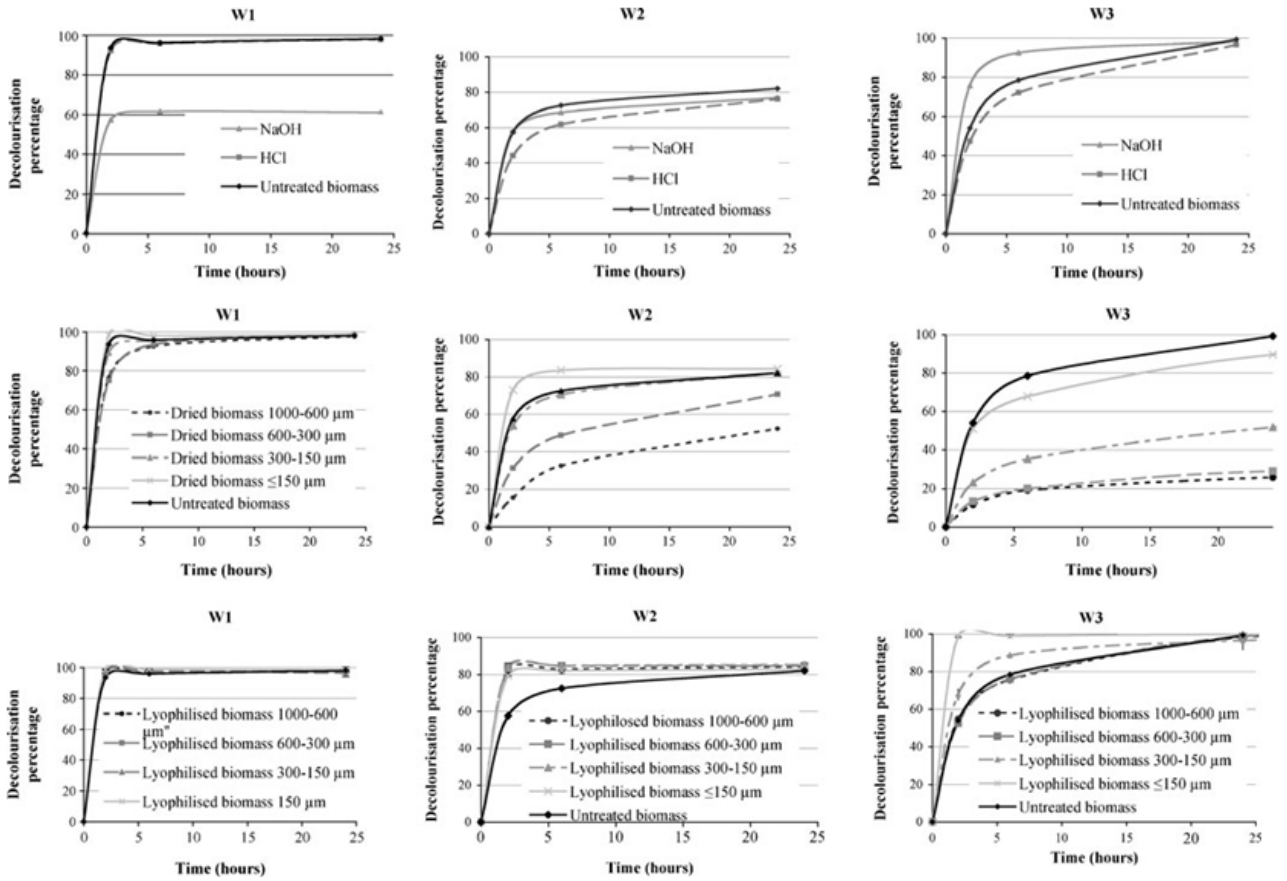


Fig. 4 Decolourisation percentage of W1 (acid spent dye bath), W2 (reactive spent dye bath) and W3 (direct spent dye bath) obtained by means of biosorption treatment with chemically (NaOH and HCl) and physically (dried and lyophilised) pre-treated biomasses of *C. elegans*

treated biomass, followed by untreated and alkali pre-treated biomasses.

Physically pre-treated biomass effectiveness

The wastewater DP values achieved by physically pre- treated biomasses and the untreated biomass of *C. elegans* are reported in Fig. 4. At the end of the experiment with W1, there were no significant differences between the DPs achieved by the physically pre-treated biomasses and that of the untreated biomass (DPs ranged from 96% to 99%). The process was very fast since more than 76% of the final decolourisation was achieved within 2 h. Nevertheless, the lyophilised biomasses determined a more rapid decolourisation (+5%) with respect to the untreated biomass, irrespectively by the particle size, whereas dried biomasses generally determined a slower decolourisation compared to the untreated biomass, and the particle size significantly affected the process.

Table 1 Biomass sorption capacity (Q_e)

Biomass		W1	W2	W3
Untreated biomass		17.5 ± 0.1Ab	251.4 ± 7.8Baef	182.7 ± 0.2Ca
HCl		10.8 ± 0.2Ac	228.1 ± 11.7Bb	173.9 ± 4.4Cb
NaOH		17.5 ± 0.2Abd	226.9 ± 4.3Bb	173.9 ± 0.5Cbgh
Dried biomass	1,000–600	17.8 ± 0.0Ad	157.4 ± 2.2Bc	46.4 ± 3.3Cc
	600–300	17.7 ± 0.1Ad	210.0 ± 2.9Bd	52.3 ± 1.5Cd
	300–150	17.7 ± 0.1Ad	245.3 ± 0.3Ba	93.1 ± 1.3Ce
	≤150	17.6 ± 0.2c	250.8 ± 1.5Be	161.2 ± 0.9Cf
Lyophilised biomass	1,000–600	18.0 ± 0.1a	249.4 ± 5.7Bf	176.5 ± 1.1 Cg
	600–300	17.5 ± 0.2bc	253.5 ± 2.9Bf	176.9 ± 1.3Cgh
	300–150	17.8 ± 0.0b	252.4 ± 1.4Bf	173.0 ± 9.1Cgh
	≤150	17.5 ± 0.1Ab	267.1 ± 7.9Bg	178.0 ± 1.2Ch

Uppercase letters indicate significant differences between Q_e of the same biosorbent towards different effluents (Mann–Whitney test, $P \leq 0.05$); lowercase letters indicate significant differences between Q_e of different biosorbents towards the same effluent (Mann–Whitney test, $P \leq 0.05$)

Table 2 COD variation percentage and toxicity reduction of W1, W2 and W3 after biosorption by means of untreated and pre-treated biomasses

Biomass pre-treatment		COD variation (%)			Toxicity reduction (%)		
		W1	W2	W3	W1 (33.3% dose)	W2 (2.5% dose)	W3 (22.2% dose)
Untreated biomass		+19.7	−49.0	−72.0	94.7	54.6	96.0
HCl		−16.5	−52.3	−78.1	78.7	62.4	100.0
NaOH		+53.5	−58.5	−61.9	57.1	89.1	42.6
Dried biomass	1,000–600	+35.5	+9.3	−1.4	37.8	48.2	55.0
	600–300	+40.0	+1.5	−0.8	42.8	53.3	67.0
	300–150	+82.9	−8.5	−17.1	63.6	59.2	80.0
	≤150	+103.2	+7.0	−7.0	75.9	74.8	84.4
Lyophilised biomass	1,000–600	+288.3	−25.7	−49.6	3.4	68.2	78.9
	600–300	+274.8	−20.3	−46.8	42.8	71.2	75.0
	300–150	+272.5	−11.1	−26.0	42.6	67.9	78.0
	≤150	+186.7	−6.7	−25.5	49.1	65.2	85.0v

Towards W2 and W3, lyophilised biomasses achieved quite always significantly higher DPs (up to 85% and 98%, respectively) than the untreated one (82% and 54%, respectively). Towards W3 only, lyophilised bio- mass particle size affected the final DP and there were significant differences among DPs at

2 h and 24 h. On the contrary, particle size of dried biomasses significantly affected both the process rapidity and the final DP towards W2 and W3.

The Q_e of physically pre-treated biomasses towards wastewaters are reported in Table 1. The drying process decreased the biomass sorption capacity towards all the wastewaters. Besides, the Q_e was dependent by the particle size towards W2 and W3. The lyophilisation determined higher biomass Q_e with respect to the untreated one only towards W2. The Q_e was quite always independent by the particle size.

Treatments with physically pre-treated biomasses pro- duced a variation in COD, although not proportional to DP (Table 2): COD values of W1 were always enhanced, whereas COD values of W2 and W3 were generally decreased (up to −25% and −50%, respectively), but less than the untreated biomass (−72%).

The pre-treated biomasses showed higher detoxifica- tion yields than the untreated biomass only towards W2. In the case of the dried biomass, the toxicity reduction was always proportional to the particle size, reflecting the trend of decolourisation, whereas lyophilised biomass size affected toxicity reduction only in the case of W1 (Table 2).

Biomass characterisation after the biosorption experiments

After biosorption experiments, the FTIR spectra of the biomasses showed more or less drastic changes depend- ing on the effluent composition. Upon immersion in W2, which has a high content of neutral salts, the spectral profile of the biomass showed a couple of new strong absorptions falling in the 1,200–1,100 cm^{-1} and 650–600 cm^{-1} ranges (Fig. 1e). These bands can be attributed to the stretching and bending modes of the sulphate group. Their presence points out that a significant amount of sulphate present in the W2 was adsorbed onto the biomass together with dyes.

Finally, all biomasses contacted with W2 showed a couple of novel and sharp endothermic transitions at 254 °C and 293 °C (Fig. 2e). This can be attributed to the interaction between the adsorbed sulphate and the organic biomass components.

Discussion

In this work, we tested the effect of chemical and physical *C. elegans* biomass pre-treatments in order to optimise the dye biosorption process in textile wastewater treatment. Actually, the method of biomass preparation can affect the biosorbent surface properties and, conse- quently, its performance (Prigione et al. 2008a). In particular, chemical and physical pre-treatments can cause (1) the removal of the outer cell wall layer, (2) the loss of cell integrity with a consequent increase of the surface area, (3) the increase in cell wall porosity with a higher exposure of its latent sites, (4) the partial loss of hyphal lumen components, which in turn increases the ratio between the cell wall and total biomass (Polman and Breckenridge 1996; Gallagher et al. 1997; Fu and Viraraghavan 2002).

The FTIR analysis highlighted that chemical pre- treatments affected the composition of the fungal biomass. Actually, besides changing the ionisation state and the H bonding arrangement of polar and ionisable chemical groups, they decreased the content of proteins and/or polysaccharides. In particular, alkali extracted important components of the fungal cell wall, mainly proteins but also a minor polysaccharide fraction, thus leaving a biomass enriched with the functional groups of other components such as the alkali-insoluble chitin and chitosan, which are extracted from the fungal cell wall by strong acid (Ruiz-Herrera 1992).

Physical pre-treatments did not bring significant effects on the intrinsic chemical composition of the material since no changes in the main absorption peaks were found. On the other hand, lyophilisation and oven drying affected the moisture content, the state of bound water, the arrangement and strength of

inter- and intra- molecular H bonds, and the physical texture of the biomasses, as demonstrated by the accumulated FTIR, DSC and TG results.

DSC and TG analyses provided evidence of the complex physical and chemical structure of the biomass. The behaviour of the main thermal transitions and the trend of weight loss were strongly influenced by the physical and chemical pre-treatments. The weight loss of the lyophilised biomass was more gradual than that of the oven-dried biomass, indicating a higher degree of homogeneity of the sample texture. Interestingly, the amount of bound water was lower in the dried biomass, but its binding strength was higher. The drying process likely allowed the different organic components of the biomass to reach a more compact and/or interconnected texture organisation, a situation that was not achieved by freeze drying. These different structures can affect the hydrophobic/hydrophilic balance of the biomass, which is one of the most important characteristics of a good biosorbent (Gadd 1993). The higher intensity of the FTIR bands attributed to the lipid components in dried biomass as compared to the lyophi- lised one is an indirect proof of the different state taken by cell wall components in response to different physical treatments. Actually, contact angle measurements pointed out that lyophilised biomass is more hydrophilic than the dried one (data not shown).

Alkali pre-treated biomass accelerated the decolourisation of W3 but worsened the W1 decolourisation; on the contrary, acid pre-treated biomass did not affect the W1 decolourisation but worsened that of W2 and W3. Never- theless, at the end of the experiment, *C. elegans* untreated biomass showed the highest biosorption capacity than the pre-treated ones, confirming the available data on dye Biosorption yield by means of chemically pre-treated biomass of other species (Bayramoglu et al. 2006).

The significant decrease of the DP and Qe yields of the alkali-treated biomass towards W1 can be due to the loss of the protein fraction, which is known to have high affinity for acid dyes under acidic conditions (Aspland 1993). Removal of these cell wall components has probably decreased the number of binding sites for acid dyes, thus resulting in lower decolourisation and sorption capacity. Accordingly, the accelerated adsorption of direct dyes from W3 by the alkali pre-treated biomass has probably been favoured by the enriched polysaccharide fraction, which has higher affinity for this class of dyes (Blackburn 2004). Actually, since amide, carboxyl and hydroxyl groups play an important role in dye biosorption, their modification is expected to decrease the effectiveness of the fungal biomass in removing dyes (Das et al. 2008).

Concerning the physical pre-treatments, dried biomass decolourisation percentage was lower than that of the untreated biomass. Moreover, it was strongly affected by biomass size. On the contrary, the lyophilised biomass achieved a similar DP with respect to the untreated one but more rapidly and generally irrespective of the biomass size.

Probably, the lyophilised biomass has higher porosity than the dried one, and this increased the contact surface with water, overcoming the influence of the biomass particle size and determining a fast re-moisturising that resulted in a more rapid decolourisation of the wastewaters. On the other hand, this physical pre-treatment is more expensive than the drying process, and this aspect should be considered in a feasibility study since fungal biomass should be produced in large scale.

Dried and lyophilised granular biomasses may help overcome conservation, robustness and separation issues (Aksu and Çağatay 2006). Moreover, the lyophilised biomass also makes biosorption process quickest, facilitating the treatment of large volumes of effluents. Thus, these results are very important from an applicative point of view because it allows overcoming some difficulties in the industrial exploitation of biosorption. Actually, although several dead biomass-based systems have been evaluated at pilot scale, none has been significantly commercialised because suspended biomass is not effective and durable in repeated long-term application, and it also makes difficult the post-separation of suspended biomass from the treated effluent (Liu and Liu 2008; Gadd 2009).

The ionic strength is an important limiting parameter for dye adsorption: both ion competition and electrical

repulsion due to salt have a fundamental role in reducing sorption capacity of dyes (Punjongharn et al. 2008). Probably, in our case, salts (70 g l^{-1}) contained in W2 significantly affected the biomass–dyes interaction, filling some biomass binding sites. Actually, FTIR analyses of coloured biomasses confirmed the biomass capability to adsorb salts. DSC thermograms and TG curves were also sensitive in detecting the effect of large amounts of adsorbed sulphate salts on the thermal behaviour of biomasses exposed to effluent W2. In W3, the high ionic strength seems to determinate an electrostatic interaction between salt and dye molecules, which results in a slower dye adsorption; actually, direct dyes in solution never reached the equilibrium with those adsorbed on all the used biomass. On the contrary, the lower ionic strength of W1 did negligibly affect the dye adsorption, as demonstrated comparing the removal of dyes from W1 in presence and absence of salt (Tigini 2010).

In addition to the pollutant removal, COD tended to decrease with the alkaline effluents W2 and W3, irrespec- tive of the biomass characteristics, while it increased to various extents with the acidic effluent W1. In particular, the alkali-treated biomass allowed achieving the highest reduction of COD in the biosorption experiment with effluent W2, and one of the highest with effluent W3, probably because no release of alkali-soluble biomass components occurred under the alkaline pH conditions of the effluents and/or chemical components contributing to COD were effectively adsorbed. Accordingly, the acid-treated biomass was the only one contributing to the decrease of COD under the acidic conditions of effluent W1.

The *P. subcapitata* toxicity test showed a significant reduction of wastewater toxicity after biosorption treat- ments. The toxicity reduction was proportional to decol- ourisation process of W2 and W3. This did not occur for W1, whose detoxification yields were proportional to size of both dried and lyophilised biomasses, despite the absence of significant differences among decolourisation percentages. Probably, this apparent inconsistency may be explained by different biomass sorption capacity of salts or other substances present in dye powders according to its particle size.

In conclusion, chemical pre-treatments of *C. elegans* biomasses can optimise the biomass affinity towards different dyes, but in our opinion the advantages of improved biosorption yields do not justify the increase in costs and environmental impact due to the use of additional chemicals for biomass pre-treatment. Among the physical pre-treatments, the lyophilisation accelerated the dye bio- sorption irrespective of biomass size, as well as allowing the biomass conservation, the robustness improvement and an easier separation of the biomass from the liquid phase. Thus, considering all these aspects, the lyophilisation is suggested for the optimisation of the fungal biomass for biosorption.

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