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Article

Fungal Waste-Biomasses as Potential Low-Cost Biosorbents for Decolorization of Textile Wastewaters

Valeria Prigione, Irene Grosso, Valeria Tigini, Antonella Anastasi and Giovanna Cristina Varese *

Department of Life Science and Systems Biology, University of Turin, Viale Mattioli, 25,
Turin 10125, Italy; E-Mails: valeria.prigione@unito.it (V.P.); irene.grosso84@gmail.com (I.G.);
valeria.tigini@unito.it (V.T.); antonella.anastasi@unito.it (A.A.)

* Author to whom correspondence should be addressed; E-Mail: cristina.varese@unito.it;
Tel.: +39-011-670-5984; Fax: +39-011-670-5962.

Abstract: The biosorption potential of three fungal waste-biomasses (*Acremonium strictum*, *Acremonium* sp. and *Penicillium* sp.) from pharmaceutical companies was compared with that of a selected biomass (*Cunninghamella elegans*), already proven to be very effective in dye biosorption. Among the waste-biomasses, *A. strictum* was the most efficient (decolorization percentage up to 90% within 30 min) with regard to three simulated dye baths; nevertheless it was less active than *C. elegans* which was able to produce a quick and substantial decolorization of all the simulated dye baths (up to 97% within 30 min). The biomasses of *A. strictum* and *C. elegans* were then tested for the treatment of nine real exhausted dye baths. *A. strictum* was effective at acidic or neutral pH, whereas *C. elegans* confirmed its high efficiency and versatility towards exhausted dye baths characterised by different classes of dyes (acid, disperse, vat, reactive) and variation in pH and ionic strength. Finally, the effect of pH on the biosorption process was evaluated to provide a realistic estimation of the validity of the laboratory results in an industrial setting. The *C. elegans* biomass was highly effective from pH 3 to pH 11 (for amounts of adsorbed dye up to 1054 and 667 mg of dye g^{-1} biomass dry weight, respectively); thus, this biomass can be considered an excellent and exceptionally versatile biosorbent material.

Keywords: biosorption; *Cunninghamella elegans*; fungi; textile industry wastewater; waste-biomass

1. Introduction

Control of pollution is one of the prime concerns of society today, since in both developing and industrialized nations a growing number of contaminants enter water supplies from human activity [1]. Actually, many industries, such as textile, paper, plastics and dyestuffs, consume substantial volumes of water, using chemicals during manufacturing and dyes to colour their products. As a result, a considerable amount of polluted wastewater is generated, which is a major source of aquatic pollution and can cause considerable damage to the receiving waters if the discharge is not adequately treated [2]. Indeed, many synthetic dyes are toxic, mutagenic, carcinogenic and represent a potential health hazard to all forms of life [3]. Prior to their release, therefore, coloured wastewaters should be treated to bring their dye concentrations down to nationally permitted levels.

Virtually all known physico-chemical techniques (coagulation, adsorption, filtration, membrane separation, *etc.*), including advanced oxidation processes (AOPs), photolysis by UV irradiation and sonolysis by means of ultrasound exposition have been explored to remove these toxic compounds from wastewater, but all of them present some drawbacks: excessive chemical usage, expensive plant requirements, high operational costs, lack of effective colour removal and sensitivity to variable wastewater input [4]. A single, universally applicable end-of-pipe solution appears to be unrealistic, and the combination of traditional and innovative techniques is deemed imperative to devise technically and economically feasible options.

Among the numerous techniques of dye removal, adsorption through activated carbons or organic resins has been so far one of the procedures of choice but the very high costs have resulted in the necessity to find alternative, cheaper adsorbent materials [2,4]. Biosorption is becoming an attractive technique thanks to its advantages over other techniques: high efficiency, cost effectiveness, and good removal performance [5]. In particular, fungi have a positive potential for the development of cost-effective biosorbents since they can be grown using unsophisticated fermentation techniques and inexpensive growth media, while producing high yields of biomass. Furthermore, many species are extensively used in a variety of large scale industrial fermentation processes where, after enzyme extraction and biochemical transformations, the biomass cannot be re-used and constitutes a waste material that is generally poorly valorised [6]. Hence, the use of waste-biomasses in biosorption application could be helpful not only to the environment, in solving the solid waste disposal problem, but also to the economy [2,7]. However, until now the potential use of fungal waste-biomasses for the removal of pollutants remains largely untapped and has been almost exclusively limited to heavy metals [6,8–11].

In the recent past, biosorption studies involving different kinds of selected organisms either dead or alive have dominated the literature. However, despite a large number of lab-scale studies on the decolorization of mono-component synthetic dye solutions, there is a need to generate relative performance data on real industrial effluents, which so far have been considered very rarely in biosorption experiments. Dye removal from real effluents should be included in studies on biosorption as this process is strongly dependent on pH, ionic strength and temperature, which are generally very variable in actual wastewaters; besides, the massive presence of salts, surfactants and other additives may hinder the dye biosorption performance [12].

In the present study, the biosorption potentials of three fungal waste-biomasses from the pharmaceutical industry (towards three simulated exhausted dye baths) were compared with that of a selected fungal biomass (*Cunninghamella elegans* Lendner) which in the light of previous experiments had already proven to be very effective in synthetic dyes and chromium removal with both mono and multi-component dye solutions, for simulated exhausted dye baths and a real tanning effluent [13–16]. Afterwards, in order to assess the value of the biosorption process under real conditions, the two most promising biomasses were tested against nine real exhausted dye baths representative of different dye types and dyeing processes. Finally, the effect of pH on the biosorption process was evaluated to provide a realistic estimation of the validity of the laboratory results in an industrial setting.

2. Results and Discussion

2.1. Decolorization

The results obtained by the biosorption tests with simulated dye baths (Figure 1) indicate that the waste-biomass of *A. strictum* provided excellent yields of decolorization of SABW (Simulated Acid Bath for Wool, 85%) and SDBC (Simulated Direct Bath for Cotton, 73%) and fair results towards SRBC (Simulated Reactive Bath for Cotton, 40%), displaying higher biosorptive capacities than *Acremonium* sp. and *Penicillium* sp. biomasses. Nevertheless, the good results obtained by *A. strictum* were lower than those obtained by *C. elegans*, which was able to effect a quick and substantial decolorization of all the three exhausted dye baths (up to 99%). Noteworthy is the fastness of the biosorption process with all the tested biomasses: in most cases the maximum yield of decolorization was obtained within the first 30 min of incubation. Only in the case of SDBC, for *C. elegans* and *A. strictum* was observed a significant increase between 30 min and 24 h.

Since different chemical groups of the fungal cell wall, such as carboxyl, amine, imidazol, phosphate, sulphydryl, sulphate, hydroxyl and the lipid fraction, have been suggested as potential binding sites [7], these different biosorption yields could be due to the different composition of the cell wall. Actually, there are marked differences in the structure and composition of the cell wall of fungi belonging to different classes such as Zygomycetes *C. elegans* and Ascomycetes *A. strictum* [17]. In particular, the main difference seems to be the extremely abundant presence of chitosan in the cell wall of Zygomycetes which is not present in Ascomycetes as *A. strictum*, and which is known to play a key role in the biosorption process [2]. Moreover, it has been reported that the culture medium (amount and type of C and N sources) and conditions (*i.e.*, fermentation process, static or agitated conditions, *etc.*), may affect the quali-quantitative composition of the cell wall [18]. In particular, Tigini [19] has recently highlighted by FT-IR analysis that *C. elegans* grown on different culture media can have great variation in the composition of its cell wall and the same *C. elegans* biomass used in the present study showed a high chitin and chitosan content.

The q_e values obtained from the tested biomasses (Figure 1), particularly those of *C. elegans* and *A. strictum*, are in line with the best results reported in the literature [5,12], but an added bonus stems from the fact that these exhausted dye baths are prepared mixing several commercially important industrial dyes which contain high concentrations of salts at different pH values, introducing real parameters that often bar the attainment of good biosorption yields [5]. It must be borne in mind that

until now most of the data concerning the exploitation of fungal biomasses in dye biosorption have been obtained on single molecules at low concentrations and only a few studies with multicomponent dye solutions and high salt concentration have been carried out [13,14].

Figure 1. (**a**–**c**) Decolorization percentage of the simulated exhausted dye baths (SABW, SRBC, SDBC) after 30 min, 2 h, 6 h and 24 h incubation by the biomasses of *Acremonium* sp., *Acremonium strictum*, *Penicillium* sp. and *Cunninghamella elegans*; (**d**) amount of adsorbed dye (q_e), letters indicate significant differences among q_e of different biomasses for the same dye bath.

(**a**)

(**b**)

(**c**)

(**d**)
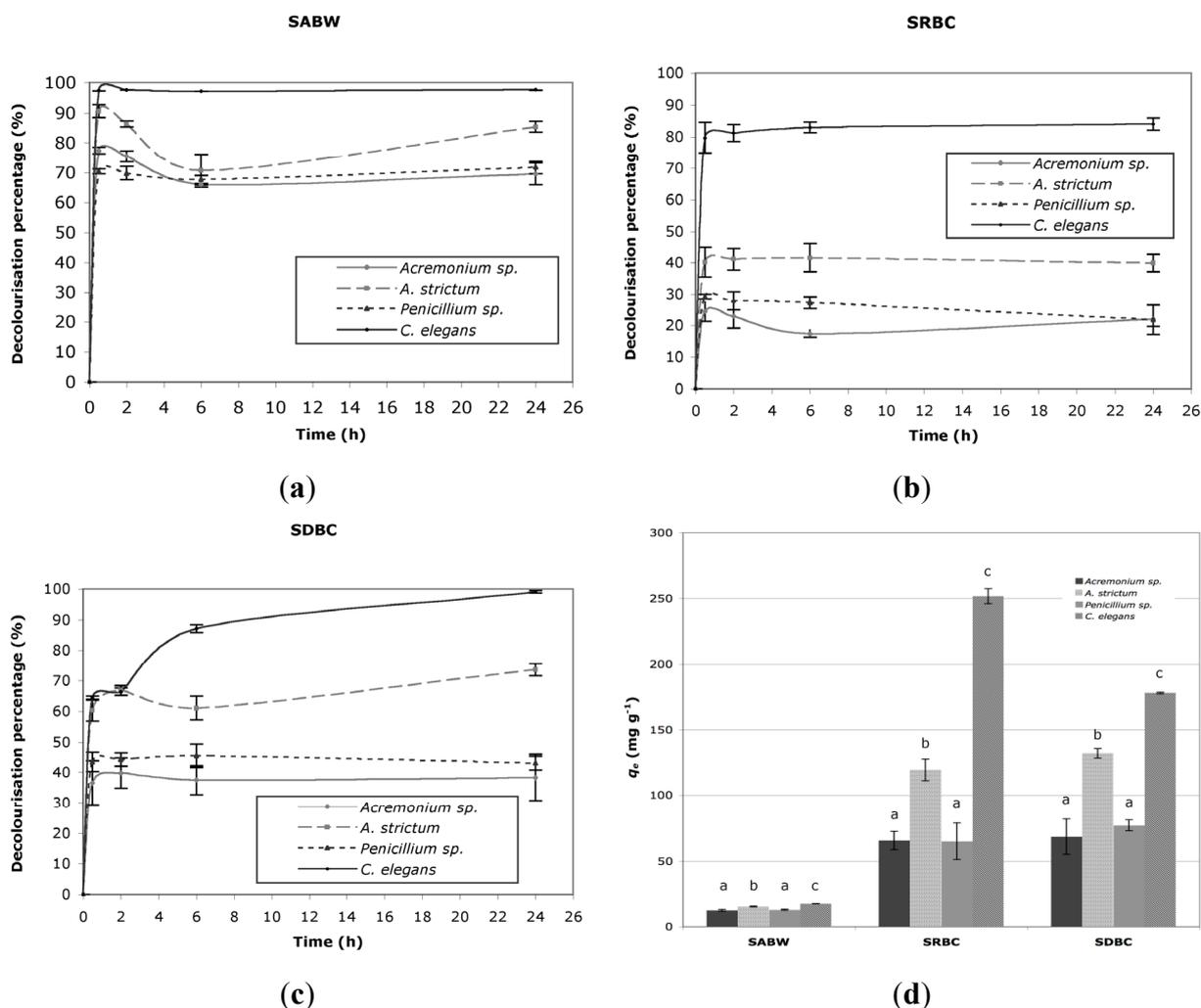
In order to compare the sorption performance of *C. elegans* and *A. strictum* biomasses, the Langmuir and Freundlich isotherms were calculated for SABW, the exhausted dye bath for which the highest *DP* values were obtained (Table 1).

The comparison of the R^2 values showed that, in both cases, the Langmuir model fits better with the experimental data than the Freundlich one. For both the biomasses, the isotherms were positive, regular and concave to the concentration axis, indicating an increase of dye uptake with an increase in the equilibrium dye concentration (Figure 2). See Table 2 for abbreviations used in the following Figures and Tables.

Table 1. Langmuir and Freundlich isotherm constants for the biosorption of SABW by *Cunninghamella elegans* and *Acremonium strictum* biomasses.

Species	Langmuir			Freundlich		
	q_{max} (mg g^{-1})	K_L (L mg^{-1})	R^2	K_F [mg$^{(n-1)/n}$ L$^{1/n}$ g^{-1}]	n	R^2
Cunninghamella elegans	594.4 ± 11.82	0.0048 ± 0.0005	0.993	54.29 ± 2.14	3.13	0.946
Acremonium strictum	289.5 ± 5.35	0.0114 ± 0.0012	0.982	48.29 ± 9.32	3.99	0.913

Figure 2. Comparison of the experimental equilibrium data with the estimated Langmuir isotherms of SABW obtained by *Cunninghamella elegans* and *Acremonium strictum* biomasses.
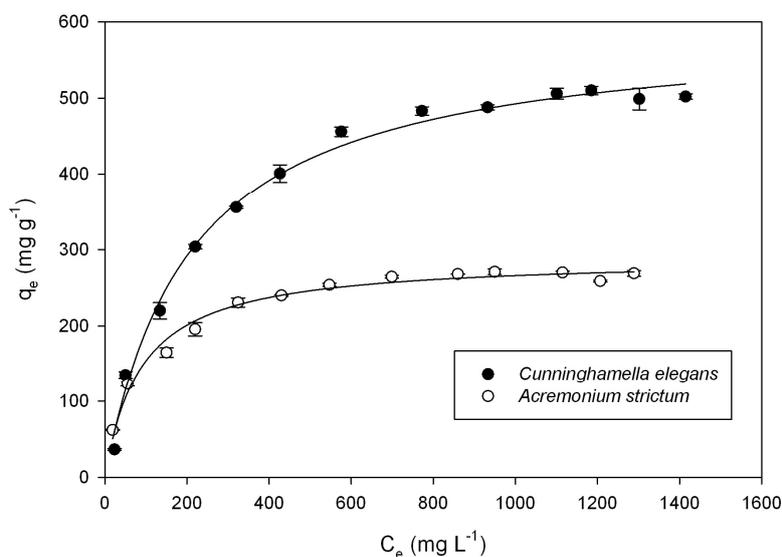
From a theoretical point of view, *C. elegans* has proven to be able to absorb a quantity of dye twice that of *A. strictum* (q_{max} 594 *vs.* 289 mg g^{-1}). Nevertheless, the high yields obtained with the *A. strictum* biomass are particularly relevant considering that to date there are very few references for the use of fungal waste-biomasses for the treatment of colored wastewaters. This biomass was more effective than the *Trichoderma harzianum* mycelia used to remove Rhodamine 6G from aqueous solution [20] or the industrial biomass of *Corynebacterium glutamicum*, tested for the treatment of a solution containing the dye Reactive Black 5 [21]. Certainly, the main advantage of the industrial waste-biomasses is the fact that they are cheap; however, the impact on costs of the multiple washings necessary to eliminate the residues of the industrial processes on the biomass that could negatively affect the decolorization yields should not be underestimated.

In order to validate a future real application in the textile industry of the biosorption process, *C. elegans* and *A. strictum* biomasses were tested for the treatment of nine real exhausted dye baths for natural and synthetic fibers (Figure 3).

Figure 3. Decolorization percentage of the real exhausted dye baths after 1 h, 2 h, 6 h and 24 h incubation by the biomasses of (**a**) *Cunninghamella elegans* and (**b**) *Acremonium strictum*.

(**a**)   (**b**)
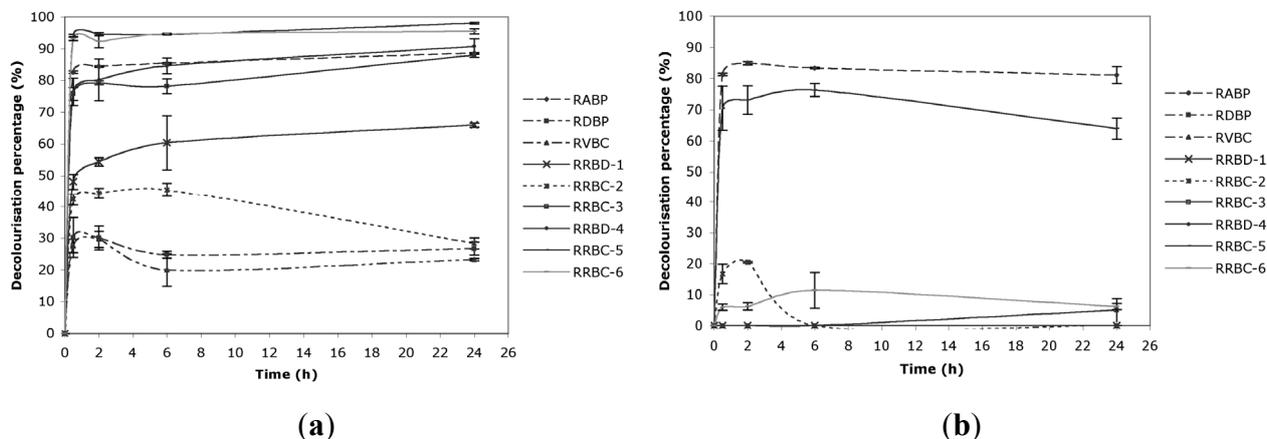
Although in the tests with simulated dye baths the results obtained by the biomass of *C. elegans* were comparable to those obtained by the biomass of *A. strictum*, in the case of real dye baths the difference between the two biomasses became substantial. The *A. strictum* biomass was only effective in the treatment of RABP and RRCB-5 (DP 81% and 64%, respectively), the two exhausted dye baths characterised by the lowest pH values. On the contrary, *C. elegans* biomass showed good yields of decolorization towards RABP and most of the reactive baths (DP up to 98%, complying to government standards), with the only exception being RRBC-2 (DP 28%); in this last case the low yield of decolorization could be due to the very high pH (11.3), the high concentration of salts (90 g L^{-1}), which may compete with the dye molecules for the same binding sites, and, probably, to the presence of a dye with low affinity for the biomass itself.

It is known from the literature that among the factors affecting the efficiency of biosorption processes, is undoubtedly the initial dye concentration: initial concentration provides an important driving force to overcome all mass transfer resistance of the dye between the aqueous and solid phases. Hence, a higher initial concentration of dye may enhance the adsorption process [5]. Nevertheless, the *C. elegans* biomass was efficient also for exhausted dye baths characterised by very low dye concentrations (e.g., RRBC-3, RRBC-4, RRBC-5 and RRBC-6).

The same biomass proved to be moderately effective against the exhausted dye baths containing disperse (RDBP) and vat (RVBC) dyes (DP 27% and 23%, respectively). These types of dye baths, however, are difficult to treat by biosorption as already stressed by other authors [22,23]. This result is probably due to the chemical characteristics of these insoluble dyes, since hydrophobic attractions, dye-dye aggregation mechanisms and dye-surfactants interactions can act simultaneously, reducing the biosorption effectiveness [2]. Moreover, in the case of disperse dyes, the presence of auxiliaries (*i.e.*, carriers), used to fix dyes to fibers, can also obstruct the dye biosorption onto the fungal biomass through competitive mechanisms [24].

The absence in the literature of works on fungal biosorption which take into account such a large number of real dye baths, differing in terms of dye types and dyeing processes, hampers a sound comparison between our data and those obtained with other fungal biomasses under similar conditions; however based on these results, the *C. elegans* biomass confirms its high efficiency and versatility for exhausted dye baths characterized by different classes of dyes, pH and ionic strength. On the other

hand, the *A. strictum* biomass proved to be effective only against exhausted dye baths at acidic or neutral pH, but it should be noted that the *A. strictum* biosorption yields are quite comparable with those of other biomasses reported in the literature [11–19]. Once again, the *C. elegans* biomass can be considered a truly exceptional biosorbent in terms of performance and versatility, however also the *A. strictum* biomass could find future application for the treatment of acid exhausted dye baths. It should also be considered that the great variability of real textile wastewaters is certainly a limiting factor for many purification technologies now available, such as biological treatment with activated sludge or adsorption with activated carbons. In the first case the sudden change in operating conditions may cause an obvious decrease in degradative activity, in the latter case, pH is the main factor limiting the yields of adsorption, especially under conditions of high alkalinity.

2.2. Effect of Initial pH

The literature indicates pH as being one of the most important abiotic parameters in regulating the biosorption yields, regardless of the sorbent material used. Actually, pH can influence the interaction between adsorbent and solute in aqueous medium in two main ways: (i) by changing the ionization potential of the dye molecules; (ii) by changing the net charge of active sites of the adsorbent surface [25]. Most of the studies on dye biosorption have reported the necessity of strong acidic conditions for optimum biosorption [26]; however, actual textile wastewaters are generally basic and adjusting and maintaining extreme acidic conditions usually increases the overall process cost [21].

The q_e values of *C. elegans* and *A. strictum* biomasses towards SABW at different initial pH values (from pH 3 to pH 11) are shown in Figure 4. In all cases the *C. elegans* biomass displayed significantly higher q_e values than that of *A. strictum*. Both the biomasses showed the highest q_e values at pH 3 (103 and 63 mg g^{-1} for *C. elegans* and *A. strictum*, respectively). From pH 5 to pH 9 *C. elegans* biomass displayed q_e values still very high (up to 89 mg g^{-1}), although significantly lower than at pH 3; whereas in the case of *A. strictum* the q_e value fell by 50% from pH 3 to pH 5. At pH 11 a significant reduction of q_e was registered with both the biomasses, which was particularly evident for *A. strictum*.

In order to evaluate the effect of initial pH of the exhausted dye bath on the sorption potentials of *C. elegans* biomass until its complete saturation, biosorption experiments were conducted in batch mode in subsequent cycles. The biomass saturation was reached at pH 3 after 12 cycles at 300 ppm and 7 cycles at 900 ppm, at pH 5 after 20 cycles at 300 ppm and 9 cycles at 900 ppm, at pH 7 after 28 cycles at 300 ppm and 9 cycles at 900 ppm, at pH 9 after 28 cycles at 300 ppm and 11 cycles at 900 ppm, and finally at pH 11 after 10 cycles at 300 ppm and 9 cycles at 900 ppm.

The total amounts of adsorbed dye (q_{tot}), obtained by summing the values of q_e of each cycle, are reported in Figure 5. The highest q_{tot} values were observed at pH 3 (1035 mg g^{-1}), pH 7 (930 mg g^{-1}) and pH 9 (945 mg g^{-1}), followed by pH 5 (824 mg g^{-1}) and, finally, pH 11 (667 mg g^{-1}).

Figure 4. Amount of adsorbed dye at the equilibrium (q_e) of *Cunninghamella elegans* and *Acremonium strictum* biomasses towards SABW at different initial pH. Capital letters indicate significant differences for *C. elegans* biomass at different pH; small letters indicate significant differences for *A. strictum* biomass at different pH.
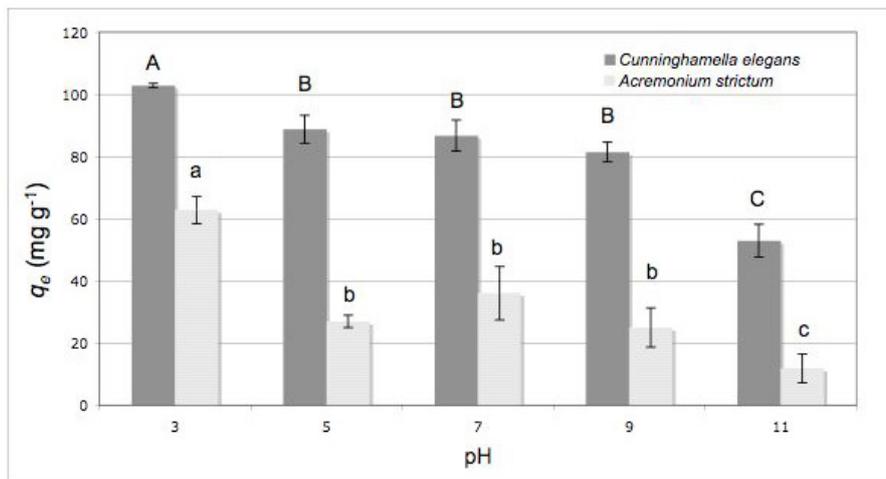
Figure 5. Total amount of adsorbed dye (q_{tot}) of *Cunninghamella elegans* biomass towards SABW at different initial pH. Letters indicate significant differences.
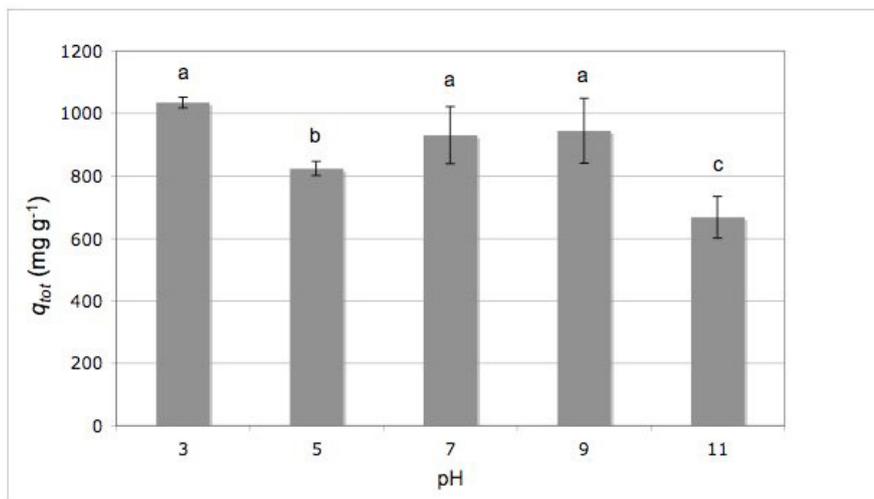
C. elegans biomass is, hence, highly effective over a wide pH range (3–11). This finding is particularly relevant in view of practical application for the treatment of real industrial wastewaters, which are often characterized by very high pH values (up to 12–13) which are of course always fluctuating. The relevance of our results is even more evident when they are compared with the literature data regarding different sorbent materials. Thus, for example, the biomass of bacterium *C. glutamicum* displayed the highest biosorption yield towards the dye Reactive Black 5 at pH 1, with an evident decrease at higher pH values [21]; similarly, the biosorptive potential of activated sludges towards Direct Black 38 drastically decreased by increasing the pH from 1 to 11 [27]. Among the fungal biomasses tested so far, *C. elegans* proved to be the most versatile with respect to this parameter: Kiran and collaborators [28], treating the dye Acid Red 57 with the biomass of *Cephalosporium aphidicola*, noting that q_e decreased proportionally to 0 with the increase of initial pH

between 1 and 6. Aksu and Çağatay [29], studying the *Rhizopus arrhizus* biomass for the removal of the dye Germano Turquise Blue-G, observed the maximum q_e at pH 2 and total ineffectiveness at pH 4. Likewise, Khambhaty and collaborators [30] using *Aspergillus wentii* to remove Brilliant Blue G observed an evident and constant q_e decrease when the solution was increased from pH 2 to 10.

Moreover, the obtained results show that the biomass can be subjected to numerous biosorption cycles until complete saturation, reducing in this way the amount of waste generated by the fungal treatment. The exhausted biomass could then be treated by incineration.

3. Experimental Section

3.1. Industrial Waste-Biomasses and Selected Test Organisms

Three waste-biomasses from industrial pharmaceutical productions were kindly provided by Antibioticos S.p.a. (*Acremonium* sp. and *Penicillium* sp.) and ACS Dobfar S.p.a. (*Acremonium strictum*, synonym *Cephalosporium acremonium*). The waste-biomasses were obtained from the pharmaceutical factories as inactivated slurries (autoclaved at 121 °C for 30 min), as required by safety procedures.

C. elegans (MUT 2861) was obtained from the *Mycotheca Universitatis Taurinensis* Collection (MUT, University of Turin, Department of Life Science and Systems Biology). It was patented for dye biosorption [31] and deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). Starting cultures of this fungus were lyophilised until use. They were revitalised on MEA and mature conidia for the inocula and biomass production were obtained from cultures grown on the same medium in the dark at 24 °C for one week.

3.2. Fungal Biomass Preparation

The waste-biomasses were rinsed in distilled water by centrifugation (9 cycles at 8000 rpm for 5 min) in order to eliminate the metabolites produced during fermentation and other colored impurities. The *C. elegans* biomass was produced according to Prigione *et al.* [15]. All the biomasses were lyophilised (Lyophiliser LIO 10P, Cinquepascal, Trezzano s/n, Italy) and powdered to particles of uniform size (300 μm < Ø < 600 μm).

3.3. Simulated and Real Exhausted Dye Baths

The composition and the characteristics of the exhausted dye baths used in this study are listed in Table 2. The three simulated exhausted dye baths (SABW, SRBC and SDBC), designed to mimic wastewater produced during wool or cotton textile dyeing processes, were prepared using mixing of industrial dyes at high concentrations. These simulated exhausted dye baths, previously developed by the industrial partners of the EC FP6 Project SOPHIED (NMP2-CT-2004-505899), were used with the permission of the SOPHIED Consortium. The industrial dyes used in these experiments were selected as being representative of different structural dye types, commercially important and with a wide range of applications across the textile industries. They are commercial products purchased from Town End plc (Leeds, UK), containing in addition to dye molecules which constitute the 30%–90% of the total weight also other organic molecules such as additives. These simulated exhausted dye baths mimic the

industrial ones also with respect to the presence of different salts, often in high concentrations, and for the pH values.

Table 2. Exhausted dye bath name, acronym, composition and pH.

Exhausted dye bath	Acronym	Dyes	Dye concentration	Salt concentration	Auxiliaries	pH
Simulated Acid Bath for Wool	SABW	Mix of 3 dyes (Abu62, AY49, AR266)	300 mg L^{-1}	5 g L^{-1}	n.i.	5.0
Simulated Reactive Bath for Cotton	SRBC	Mix of 4 dyes (Rbu222, RR195, RY145, Rbk5)	5000 mg L^{-1}	70 g L^{-1}	n.i.	10.0
Simulated Direct Bath for Cotton	SDBC	Mix of 3 dyes (DrBu71, DrR80, DrY106)	3000 mg L^{-1}	2 g L^{-1}	n.i.	9.0
Real Acid Bath for Polyamide	RABP	Mix of 3 dyes	433 mg L^{-1}	n.i.	Surfactants, weak acid, fixatives	5.0
Real Disperse Bath for Polyester	RDBP	1 dye	658 mg L^{-1}	n.i.	Dispersants, weak acid, strong base	9.3
Real Vat Bath for Cotton	RVBC	Mix of 2 dyes	1424 mg L^{-1}	20 g L^{-1}	Weak acid, strong base, glucose	12.7
Real Reactive Bath for Cotton 1	RRBC-1	Mix of 3 dyes	542 mg L^{-1}	77 g L^{-1}	Weak acid, strong base	10.9
Real Reactive Bath for Cotton 2	RRBC-2	1 dye	343 mg L^{-1}	90 g L^{-1}	Ca and Mg sequestering, oil, weak acid, strong base	11.3
Real reactive Bath for Cotton—continuous dyeing	RRBC-3	Mix of 3 dyes	43 mg L^{-1}	200 g L^{-1}	Sodium carbonate	10.3
Real reactive Bath for Cotton—washing with cold water	RRCB-4	Mix of 3 dyes	34 mg L^{-1}	200 g L^{-1}	n.i.	10.0
Real reactive Bath for Cotton—neutralization and washing at 40 °C	RRCB-5	Mix of 3 dyes	98 mg L^{-1}	200 g L^{-1}	Acetic acid	5.9
Real reactive Bath for Cotton—boiling soaping	RRCB-6	Mix of 3 dyes	60 mg L^{-1}	200 g L^{-1}	Surfactants	7.8

Note: n.i. means not indicated in the dye bath formulation.

The nine real industrial exhausted dye baths used in this study were kindly provided by textile industry members of the BIOTEX Project (Call MD 2007 to promote excellence in the Lombardy Region meta-districts) and are representative of different dye types (acid, disperse, reactive, vat) and dyeing processes (batch and continuous). The exhausted dye baths RABP, RDBP, RVBC, RRBC-1

and RRBC-2 were taken from batch dyeing plants, after the dyeing process, while RRC-3, RRC-4, RRC-5 and RRC-6 all coming from a continuous dyeing plant, were collected at four stages of the process (*i.e.*, after dyeing, washing with cold water, neutralization/washing at 40 °C and boiling and soaping). The dye concentration was obtained using a sample of the dye bath at known concentration, taken before the dyeing process: the absorbance spectrum area of this sample was compared with that of the same bath taken after the dyeing process, in this way it was possible to calculate, indirectly, the residual dye concentration in the exhausted baths.

3.4. Sorption Experiments

Each biomass was weighed and 0.5 g dry weight was placed in a 50 mL Erlenmayer flask containing 30 mL of simulated or real exhausted dye bath. The flasks were incubated at 30 °C under agitated conditions at 130 rpm. Each trial was performed in triplicate. Exhausted dye baths without biomass were used as abiotic controls to assess decolorization other than that due to biosorption (e.g., photobleaching or complexation).

After 30 min (or 1 h), 2, 6 and 24 h, 200 μL were taken from each sample of the exhausted dye baths, centrifuged at 14,000 rpm for 5 min to remove disturbing mycelial fragments, and examined with a spectrophotometer (TECAN Infinite M200, Grödig, Austria) to obtain the complete absorbance spectra. Since a linear relationship existed between the area of absorbance spectrum and dye concentration, the percentage of removed dye (*DP*, decolorization 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. At the end of the experiment the amount of adsorbed dye (q_e), that is mg of adsorbed due g^{-1} of biomass dry weight, was determined by using the following equation, taking into account the dye concentration difference in the exhausted dye bath at the beginning and at equilibrium:

$$q_e = (C_i - C_e) \cdot V/m \tag{1}$$

where C_i and C_e are the initial and the equilibrium dye concentrations (mg L^{-1}); V is the volume of the solution (L); and m is the amount of the biosorbent used (g).

The significance of differences ($p \leq 0.05$) among the *DP* values at 30 min (or 1 h), 2, 6 and 24 h and among q_e values was calculated with the Mann-Whitney test (SYSTAT 10 for windows [32]).

3.5. Adsorption Isotherms

Adsorption isotherm experiments were carried out by bringing into contact a fixed amount of biomass with a suitable volume of SABW at an appropriate concentration. The equilibrium data were obtained by investigating a wide range of concentrations, representative of the application in wastewater remediation treatment.

Adsorption isotherms were obtained by correlating the amount of adsorbed solute (q_e) with the residual concentration of dye in solution at equilibrium (C_e). The two following equilibrium isotherm models were used to fit the experimental data.

Langmuir model:

$$q_e = q_{max} \cdot [(K_L \cdot C_e) / (1 + K_L \cdot C_e)] \tag{2}$$

Freundlich model:

$$q_e = K_F \cdot C_e^{1/n} \tag{3}$$

where q_{max} is the maximum solid phase concentration of adsorbate (forming a complete monolayer coverage on the sorbent surface); K_L is the Langmuir constant related to the solute affinity for the sorbent-binding sites; K_F and n are Freundlich constants; K_F and slope $1/n$ are defined as a sorption coefficient representing the amount of dye molecules for a unit equilibrium concentration and as a measure of the sorption intensity or surface heterogeneity, respectively; a value of $1/n = 1$ shows that the partition between two phases does not depend on the concentration; a value of $1/n < 1$ corresponds to a normal Langmuir isotherm; while $1/n > 1$ indicates a cooperative sorption involving strong interactions between the molecules of adsorbate [33].

3.6. Effect of Initial pH

Eighty-four mg of lyophilised biomass (corresponding to 0.5 g of biomass fresh weight) was placed in a 50 mL Falcon tube containing 30 mL of SABW at different pH values (3, 5, 7, 9 and 11). The flasks were incubated as previously described. Once the adsorption equilibrium was reached, *i.e.*, after three consecutive measurements resulting in an equal *DP* value, the samples were centrifuged at 8000 rpm for 5 minutes, and the treated dye bath was replaced by 30 mL of the untreated one. The test ended when the biomass was no longer able to adsorb dye. Finally, the total amount of adsorbed dye (q_{tot}) was calculated by adding up the q_e values obtained at the end of each cycle.

4. Conclusions

The results obtained in this study enable the following conclusions to be drawn:

(1) *A. strictum* biomass was more efficient than the other two industrial waste-biomasses, being able to substantially decolorize the simulated dye baths; however, it was effective only towards the real ones characterized by acidic pH. Hence, at the moment, industrial waste-biomasses such as *A. strictum* can be considered competitive and potentially useful for the treatment of specific types of wastewater (e.g., acid exhausted dye baths) only;

(2) *C. elegans* biomass is endowed with a high ability to remove dyes belonging to different chemical classes, not only from simulated dye baths but also from many real ones;

(3) The high applicative potentialities of *C. elegans* biomass for the decolorization of textile wastewater was demonstrated by the very good biosorption yields even under extreme conditions of pH (3–11); for this reason, this biomass can be considered an excellent and exceptionally versatile biosorbent material.

Hence, biosorption by means of *C. elegans* biomass could be considered a valid alternative to other techniques for wastewater treatment, being applicable to real industrial wastewaters representative of different dye types (acid, disperse, vat, reactive) and dyeing processes (batch and continuous) in a timely fashion. Future investigations are obviously needed to validate the data obtained at pilot scale and to verify under field conditions the possibility to couple the biosorption process with other physical and biological/chemical treatmen, aiming at complete decolorization of effluents and water

reuse. Since biosorption is very effective towards wastewater with high concentrations of dyes, this technique seems to be particulary suitable for application as a primary treatment. Partially purified wastewater could then be sent for subsequent conventional treatment such as bioxidation by activated sludge.

Acknowledgments

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