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A Scaling-up Issue: The Optimal Bioreactor Configuration for Effective Fungal Treatment of Textile Wastewaters

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Real textile wastewaters were treated by a combined method with fungi and activated sludge. In this work, the white rot fungus *Trametes pubescens* MUT 2400 which has already showed to decolorize wastewaters, was exploited for biodegradation purposes with the aim to scale-up the system to larger working volumes. A bubble column reactor, a fixed bed reactor and a stirrer tank reactor were investigated. Colour, COD and toxicity were monitored before and after both the treatments. It was thus possible to identify the main targets towards which the two biological methods are mainly effective: colour for fungi whereas COD and toxicity for the activated sludge. Both the growth and the degradation yiedl of the fungus were highly affected by the different aeration and agitation conditions, with strong repercussion on the final process yields. Actually, DP values were very low when the bubble column reactor was used. On the contrary, the stirrer tank reactor resulted the best reactoristic configuration: the extensive decolourisation (85 %) and the COD reduction (95 %) were coupled with a significant detoxification monitored by *L. sativum* test. Consequentially, thanks to the combined biological treatment, the textile effluent parameters complied the Italian legal threshold limit for the discharge in surface waters.

1. Introduction

Textile industry is rated as one of the most polluting among all industrial sectors, due to the huge volumes of discharged wastewaters. General estimations consider that they can contain more than 2000 different chemicals including dyes, auxiliaries present in the dye formulation (dispersing agents, anti-foaming agents, etc.), basic chemicals and auxiliaries used in dyeing processes (alkali, salts, reducing and oxidizing agents, etc.) and residual contaminants coming from the fibres, such as pesticides (dos Santos et al. 2007).
Once discharged into the environment, these effluents may affect primarily but not exclusively the aquatic habitat; indeed surface waters may be used in field irrigation or processed as drinking waters, finding their way back to human society. The evaluation of innovative biological approaches is needed for water decontamination. Actually, these are among the most promising solutions, being more economically and environmentally sustainable than chemical and physical methods.
Fungi are able to transform synthetic dyes in colourless substances but it is important to get far from model conditions (Baccar et al. 2011, Gao et al. 2010). To date, very few experiments have faced real industrial problematics associated to the presence of the autochthonous microflora and high concentrations of salts, dyes, detergents and many other aromatic compounds including phenols (Blanquez et al. 2008).
Moreover, the system scale-up to larger volumes is essential to evaluate the effectiveness and the suitability of the fungal treatment, from a technological and economical point of view. Only in few cases, bioreactors have been used to assess the behaviour of fungal biomasses at larger working volumes, investigating different reactor technologies as stirrer tank, air lift, bubble column, fixed bed, membrane, rotating disk, etc. (Anastasi et al. 2010, Cerrone et al. 2011, Junghanns et al. 2012, Rodarte-Morales et al. 2012).

Each of these technologies has obviously pros and cons that highly influence the applicability at industrial scale, highlighting the importance to choose the correct configuration able to fit to the general requirements and needs of any specific process (i.e. free or immobilized biomasses, nutrients supply, time durance, presence of extracellular active metabolites, etc.). Moreover, as regards fungi, the development of fermentation technologies went just a little forward: very few is known about their needs and responses to medium feeding, agitation/aeration rate and methodology, etc, in order to minimize the interferences on hyphal growth and strengthen the fungal metabolism. Hence, the selection of the most suitable technology and the optimisation of working parameters are key factors for the development of a competitive and applicable fungal treatment. For example, the use of impellers for liquid agitation is a good option mainly when the biomass is immobilized, because of the mechanical protection provided by supports: few mycelium damages occur but the oxygenation inside the reactor is maximized.
A wrong reactor choice may lead to low yields or slow process. For example, the degradation of a tannery dye by *Trametes versicolor* was less efficient in an air-pulsed reactor than in flasks, reducing 60 % and 80 % of the colour, respectively (Baccar et al. 2011).
In the present study, the environmental issues associated to pollutant wastewaters outcoming from a textile industry was faced using a biological approach, based on a fungal treatment coupled with activated sludge. *Trametes pubescens* MUT 2400 was selected because of its already demonstrated capability to treat textile wastewaters (Anastasi et al. 2012). This system was here scaled up to larger operative volumes by means of different fermentation technologies. A bubble column reactor, a fixed bed reactor and a stirrer tank reactor were investigated, as literature data demonstrated their appropriacy for the growth and the maintenance of fungal biomasses. This choice was also driven by the collaboration with a company active in wastewater treatment, which defined these solutions applicable in its plant.

2. Methods

2.1 Strain and Effluents

Trametes pubescens MUT 2400 is preserved at the *Mycotheca Universitatis Taurinensis* (University of Torino). The fungus was grown and immobilized on polyurethane foam cubes (PUF) as described elsewhere (Spina et al. 2012).
The wastewaters were kindly provided by Fidia Engineering s.r.l. (BG, Italy) and were sampled from the homogenization tank before the activated sludge treatment. The textile effluents (TE1 and TE2) were highly coloured, strongly alkaline (pH 11.0 and 11.9) and with a COD of 278 and 364 mg/L, respectively. The pH was adjusted to a neutral value (pH 7) and, due to the low organic content, a low amount of glucose (0.1 g/L) useful for fungal sustenance was added.

2.2 Reactors

After 7 days of pre-culturing, the colonised supports were added to the textile wastewater: the same loading factor was applied to all the reactor configurations (60 PUF/L, 10 % v/v). The reactors were set as an open system and the sterility was not maintained during the treatment. Since the operative reactor parameters may influence the fungal growth and metabolism, a reference system was used: 1 L flasks run with 500 mL working volume and the same loading factor applied to reactors.
In order to simulate a real industrial process, after the fungal treatment (phase I, 48 h), the liquid was separated and inoculated with activated sludge (phase II, 48 h). The experiment were run at room temperature (22-25 °C). Periodically, 1 ml of wastewater was collected for decolourisation and enzyme activities monitoring.
Bubble Coloumn Reactor (BCR): a 5 L reactor (Biostat B plus - 2L MO, Sartorius, AG, Goettingen, Germany) was set up in order to minimize mechanical stresses (i.e. hyphal disruption) with a working volume of 2 L of TE1. No mechanical agitation was provided and the air was injected at 2 L/min from the bottom of the tank.
Fixed Bed Reactor (FBR): a glass column of 30 cm height was connected to a peristaltic pump to maintain the liquid in continuous recirculation (1 L/h). The system was packaged with 40 colonised PUF; few uncolonised supports were also placed at the head, bottom and centre of the column in order to enhance the liquid diffusion. The column was fed with 700 mL of TE2, and more than 300 mL represented the contact volume.
Stirrer Tank Reactor (STR): a 5 L reactor was loaded with 2 L of TE2. The agitation was provided by an elephant ear impeller placed at the bottom of the reactor (200 rpm). The aeration was maintained by the recirculation of the liquid phase which continuously drove the more aerated medium from the air/liquid surface to the bottom of the vessel. No additional aeration was provided since the present set up reflected the real conditions that could be created in the plant of interest.

2.3 Analysis

The absorbance spectrum of the effluent was spectrophotometrically measured (TECAN Infinite M200, Austria) in the visible range (360-790 nm): the decolourisation percentage (DP) was calculated with respect to the starting effluent spectrum.

Laccase activities were determined by following the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) in sodium-citrate buffer (pH 3) at 420 nm (ε_{420} = 36 mM^{-1} cm^{-1}) (Niku-Paavola et al. 1988). The chemical oxygen demand (COD) was evaluated by using the dichromate method (Kit Lange, COD range 100-2000 mg/L) and dissolved oxygen by means of a portable oximeter (Stereoglass Oxi 330).

The ecotoxicity of the textile wastewaters was evaluated before and after both the biological treatments: the test on *Lepidium sativum* seeds was set up according to the method UNICHIM N. 1651-2003. The germination index (GI%) was calculated according to the formula: GI% = (Gs*Ls)/(Gc*Lc)*100, where Gs is the mean number of germinated seeds in the sample, Ls is the mean root length of plantulae in the sample, Gc is the mean number of germinated seeds in the control (consisting of distilled water), Lc is the mean root length of plantulae in the control.

3. Results and Discussion

When a process is scaled up in reactor, aeration and agitation change in comparison with flasks. Fungal biomass responds differently to the mechanical and oxidative stresses, and its activity can change. In order to define the reactor able to strengthen the fungal activity, different fermentation technologies were investigated. The effectiveness of the biological treatment was evaluated, monitoring the colour, the COD and the ecotoxicity, whose control is required before the discharge into receiving waters and governed by more and more restrictive laws.

3.1 Bubble Column Reactor

A Bubble Column Reactor (BCR) has many practical advantages as the low energetic requirement: the injected air allowed both to maintain the proper content of oxygen and the homogeneity of the culture, avoiding the use of mechanical stirring. Moreover, this technology has been already applied in fungal fermentation, mostly resulting adequate to support mycelial development and activity. For example, *T. versicolor* reduced the colour of Black Dicem TTO of 60 % in 24 h, showing a complete decolourisation (97 %) within three days (Baccar et al. 2011). It was also a suitable tool for the maintenance of a stable treatment over time: *T. versicolor* continuously decolourised 70 % of Grey Lanaset G for three months and 40-60 % of a textile effluent for 15 days under non sterile operative condition (Blanquez et al. 2008).

Thereby, *T. pubescens* MUT 2400 (phase I) was highly effected by culture conditions established in the reactor: the DP values were 80 % and 30 % in flask and in BCR, respectively (Table 1 and Figure 1). The fungal treatment was always more efficient than activated sludge, which removed almost 15 % of the colour (AS alone or phase II).

As regard the COD (Table 1), the fungal treatment provoked a considerable increase of the initial value, probably due to the presence of a residual culture medium in the colonised PUF. On the other hand, activated sludge was very active towards this parameter, even in presence of the already fungal treated TE1. At the end of the treatment, the COD values were comparable to or below those at the beginning of the experiment.

The toxicity assessment was evaluated by a model test, highlighting some correspondences with DP profile: the lower the colour reduced, the higher detoxification was reached (Table 1). As already discussed, the fungus may oxidize dyes, creating colourless intermediate metabolites that could result even more toxic than the former ones (Vanhulle et al. 2008). Hence, the very high decolourisation yield obtained in flask was coupled with toxicity increase: the IG% lessened from 59 % (untreated TE1) to 4 %. The removal of 80 % of the colour may have, then, led to the transformation of chromophores in other toxic colourless molecules. Noteworthy, activated sludge was able to increase again this parameter.

Table 1: Decolourisation percentage (DP), COD values (mg/L) and index of germination (IG%) of L. sativum after the fungal treatment (phase I) and activated sludge (phase II).

	DP		COD		IG%	
	phase I	phase II	phase I	phase II	phase I	phase II
BCR	31.3	6.3	1641	318	90.0	92.3
flask	82.3	3.3	717	138	4.1	18.3
activated sludge	--	16.4	--	110	--	136.6

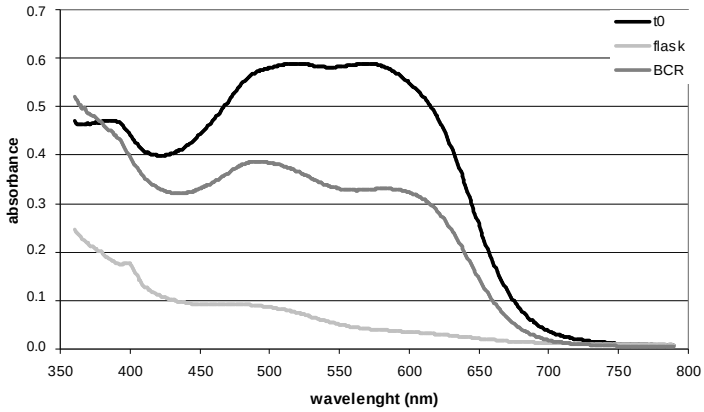

Figure 1: Absorbance spectrum of the untreated (t0) and treated TE1 by T. pubescens MUT 2400 (phase I) in flask and in BCR.

On the other hand in the BCR, the still highly coloured TE1 (DP 30 %) was almost not toxic, showing a IG% close to 100 %: the fungus transformed few chemical components but the ones which prejudiced the seed germination.
Comparing the results obtained in flask and in the reactor, BCR seemed not to be a feasible solution, without any benefit in terms of final decolourisation yield. The optimisation of the operating parameters of the bioreactor was probably needed, as well as the evaluation of different types of bioreactors, in order to define the optimal conditions that enhance the removal of pollutant compounds in the wastewater.

3.2 Fixed Bed Reactor and Stirrer Tank Reactor

According to previous results, the fungal treatment was carried out by means of other reactor solutions. Fixed Bed Reactors (FBR) has been successfully applied for lasting treatment of textile wastewater: *Bjerkandera adusta* removed 70-80 % of the colour during repeated cycles (70 days) in non sterile conditions (Anastasi et al. 2010). Stirrer Tank Reactor (STR) is usually an optimal solution to guarantee a good homogeneity of the liquid, enhancing the contact between pollutants and the mycelium (Papaspyridi et al. 2012).
As shown in Table 2 and Figure 2, *T. pubescens* MUT 2400 actively decolourised TE2 in all the tested conditions. Since all lines were set up with the same loading factor, the observed different decolourisation yields were exclusively ascribable to the operative culturing conditions.
The highest decolourisation yield (86 %) was achieved in the STR, where probably the mechanical stirring guaranteed an appropriate liquid homogeneity and oxygen transfer. Noteworthy, the fungal treatment obtained higher DP values in STR than in flask: operative aeration and agitation conditions inside the reactor resulted optimal and maximized the fungal activity. As a confirmation, the highest laccase concentration (50-100 U/L) was detected in STR, whereas in the other lines the enzymatic activity was minimal (below 20 U/L). On the other hand, oxygen transfer rate was probably limiting in FBR and the fungus removed only 30 % of the colour.
Confirming previous experiments and other experimental evidences (Spina et al. 2012), activated sludge removed very weakly the colour. Bacteria reached only 22 % of decolourisation, being always less efficient than *T. pubescens* MUT 2400.
In comparison to the control where the pH rose to 8, the fungus acidified TE2 (Table 2), probably with the final aim to keep the pH close to the optimum of oxidative enzymes: laccases have a maximal activity peak at pH 3, but they may work in a wide range of pH (2-6). On the other hand, since the metabolism of the activated sludge requires a more basic environment, the pH rose to values of about 8.5.

Table 2: Decolourisation percentage (DP), COD values (mg/L) and pH after the fungal treatment (phase I) and activated sludge (phase II).

	DP		**COD**		**pH**	
	phase I	phase II	phase I	phase II	phase I	phase II
STR	86.6	4.3	703	149	2.62	8.05
FBR	33.1	15.8	760	90	2.59	8.44
flask	44.7	10.7	608	148	5.95	8.56
activated sludge	--	22.5	--	94	--	8.61

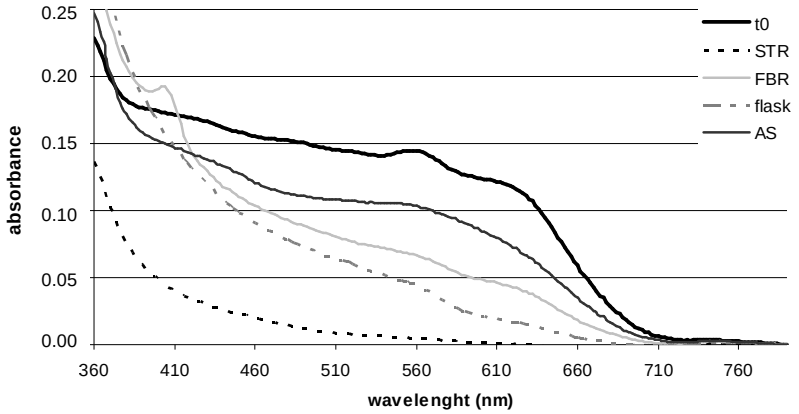

Figure 2: Absorbance spectrum of the untreated (t0) and treated TE2 by T. pubescens MUT 2400 (phase I) in flask, in BCR, in STR and by the activated sludge.

Activated sludge was particularly active towards COD, reducing the initial value (364 mg/L) of more than 75 % (Table 2). The fungus (phase I) determined the increase of COD values, probably due to residual traces of exhausted medium inside the colonised supports and fungal metabolites, including organic acids, enzymes, polysaccharides, etc, which thereby did not inhibit activated sludge. During phase II, COD values decreased and, at the end of the two biological treatments, resulted below the Italian legal threshold limit (160 mg/L).

The elevate metabolic activity displayed by *T. pubescens* MIUT 2400 was confirmed by the consumption of the oxygen dissolved in the liquid (Table 3). This value is given by the oxygen uptake from the liquid by the fungal for its own metabolism and by oxidation reactions. Along with the fungal activity, this parameter is useful to evaluate the actual oxygenation provided during different growing conditions, i.e. defining whether the aeration inside the reactor is optimal or limiting.

Since the measured data were comparable (about 5 mg/L), it could be assumed that stirrers maintained a proper inner aeration as in the flask. The oxygen consumption by the fungus in FBR increased during the experiment, but this profile could not be correlated with the time profile of the decolourisation. In agreement with data measured in the plant, activated sludge requires high amount of oxygen, and the detected dissolved fraction in TE2 was around 1-2 mg/L.

Along with colour and COD, a proper risk assessment needs also additional information about how aquatic organism respond to the presence of textile wastewaters. With this aim, ecotoxicological bioassays represent powerful tools, being able to predict the toxic effects they may cause on model organisms (Tigini et al. 2011). In the present study, *L. sativum* test was used, evaluating the index of seeds germination (IG%). As reported in Table 3, no significant differences were found among fungal cultures: at the end of phase I, they all showed an increase of the toxicity, despite the different yields of decolourisation and COD removal (Table 2).

On the contrary, activated sludge was able to consistently increase the seed IG%. Indeed the toxicity of TE2 was reduced due to the partial degradation mediated by the activated sludge of intermediate metabolites of the fungus or of the xenobiotics oxidation, as witnessed also by the COD reduction (Table 2). However, some fungal produced compounds were still left and influenced the seed germination: IG% was 109 % and 80 % when the activated sludge worked alone or after the fungal treatment, respectively.

Table 3: Dissolved oxygen (mg/L) during phase I and index of germination (IG%) of L. sativum after the fungal treatment (phase I) and activated sludge (phase II).

	oxygen			IG%	
	3 h	24 h	48 h	phase I	phase II
STR	4.96	5.41	4.25	35.1	77.3
FBR	7.13	2.64	4.32	30.1	72.8
flask	5.66	5.85	6.72	31.6	75.6
activated sludge	1.68	2.02	1.62	--	109.6

4. Conclusion

Stirrer Tank Reactor (STR) was the most suitable reactor technology for the maintenance of the activity of *T. pubescens* MUT 2400, demonstrating the adequacy of the mass and the oxygen transfer established by controlled mechanical agitation (Papaspyridi et al. 2012). In the present study, Fixed Ber Reactors (FBR) did not represent a suitable option for the fungal treatment of textile wastewaters, even though in literature many successful applications are available (Rodarte-Morales et al. 2012).
In conclusion, the fungus was particularly active towards the colour, while activated sludge acted on COD and toxicity: the two organisms operated in a synergic way towards different components of the effluent. The combination of the two approaches could be hypothesized in order to couple their potentials for a complete remediation of textile effluents. On the whole, the fungal treatment carried out in STR followed by activated sludge was able to almost completely reduce the colour, the COD and the toxicity of real textile wastewaters, complying the threshold limits for the monitored parameters, fixed by the Italian legislation of industrial effluents into receiving waters.

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