Fungal biocatalysts in the textile industry: Whole-cell systems in real textile wastewater treatment

1. Fungal biocatalysts in textile industry: whole-cell systems in real textile wastewaters treatment

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Abstract

One of the main purposes of biotechnology is to make industrial manufacturing processes more environmental friendly. In particular, treatment of textile wastewaters represents a still-open problem, being traditional systems costly and only partially effective.

In this context, fungi and their metabolites mediate oxidative reactions that may be fruitfully employed for color and toxicity reduction. To date, most of the researches have been carried out using model or axenic conditions, poorly predictive of the fungal behavior in varying and heterogeneous environments as in wastewater treatment plants.

Otherwise a great variability among fungi in terms of robustness and activity has been outlined, requiring a case-by-case optimization of those factors that may interfere with fungal activity. Due to the very harsh conditions of textile effluents, their modification is often necessary to make fungi fully active: nourishment scarcity and alkalinity might be corrected to strengthen fungal biomass.

Particular attention should be given to the technological evolution of the fungal process. Any proposed fungal treatments should carefully choose the most proper reactor configurations ensuring

the highest potentials in any specific field of application. Besides, the immobilization of the fungal biomass can provide many advantages, protecting the mycelium from external stressors and limiting its uncontrolled overgrowth. Several carriers are commercially available, but the shape, the material and the structure differ, influencing the biomass adhesion and stability over time.

Finally, a fungal technology needs to be integrated with the already existing processes, synergistically working but not interfering with them. Environmental conditions and technological apparatus in plants can be slightly modified and the optimal location for the fungus to work has to be defined.

The aim of this chapter is hence to draw a general picture of the features that a fungal treatment must have to be potentially inserted along a wastewater treatment plant. Many technological solutions will be investigated, deepening the crucial factors that may ensure its actual exploitation in plant.

Keywords

real textile wastewaters, toxicity, reactors, fungal supports, fungal degradation

1.1 Introduction

During textile manufacturing high volumes of water are consumed with the consequent production of large amount of wastewaters. Considering that dyeing process needs up to 100-200 l of water for each kg of fabric, the average annual production of 40 million tons of textile fibers causes the release of 4-8 million m^3 of contaminated effluents (Ileri et al., 2009). In the nearby future, clean water is going to acquire much more value making water saving along industrial processes a central priority: during 20 years (1990-2009), cotton manufacturing processes almost halved the average needed volumes, but more than 70 l of water are still necessary for each kg of processed fabric (Thiry, 2011).

Besides, these processes generate heterogeneous effluents containing 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 fibers, as pesticides (dos Santos et al., 2007). General estimations consider that they can contain more than 2000 different chemicals. Moreover, the transformation of dyes may be considered just a first step forward the actual decontamination of these polluted waters: the complete mineralization is rarely achieved and most of the former molecules may be still intact. As a consequence, COD values often remain unaltered even though the color is gone. Moreover, the intrinsic toxicity of transformed colorless compounds has been already recognized (Vanhulle et al., 2008a; Hai et al., 2006). Thus, color removal is not exhaustive to decrease the environmental concern, and COD and toxicity should be carefully evaluated.

The discharge of textile effluents into receiving waters poses environmental concerns in a more extended area than the sole water system closed to industrial plants. Actually they can enter into the water cycle by field irrigation or been processed and become drinking waters, giving particular emphasis to a complete risk assessment evaluation and an effective decontamination.

The chemical complexity of textile effluents has led the onset of multi-phases wastewater treatment plants (WWTPs). Traditional techniques often result only partially effective because most of these

compounds are highly resistant to physical, chemical or biological treatments. Tertiary treatments are usually necessary to comply the threshold limits but they are quite expensive, consume a lot of energy and involve potentially toxic compounds. Many efforts have been done to clean up textile wastewaters by optimizing existing technologies or combining them with innovative cost-effective biological approaches.

Fungi, in particular white rot fungi, have long been recognized for their abilities to transform a broad range of recalcitrant compounds through the use of non-specific extracellular oxidative enzymes as laccases and peroxidases (Kaushik and Malik, 2009; Gao et al., 2010). Degradation capabilities are variable among strains, due to the physiological and genetic differences.

Factors governing dye decolorization by fungi in controlled conditions have been deeply discussed and described (Kaushik and Malik, 2009; Gao et al., 2010). Accordingly, this chapter is centered on real textile wastewaters treatment: along with the description of the fungal potential and the actual problems that fungi should face in real WWTPs, the strategies outlining a winning treatment will be deepened.

1.2. Fungal degradation in axenic conditions

Many evidences have demonstrated that fungi are able to degrade synthetic dyes representative of the two main chemical dye groups (azo and anthraquinonic), as Orange G and RBBR (Novotny et al., 2001; Jarosz-Wilkołazka et al., 2002; Anastasi et al., 2010a; Anastasi et al., 2011). This process seems to involve different extracellular enzymatic patterns. For example, *Pleurotus calyptratus* and *Ischnoderma resinosum* decolorize Orange G by means of laccases, whereas peroxidases are mainly involved in RBBR transformation (Eichlerova et al., 2006).

To date, most of the researches have used synthetic effluents in controlled conditions, working with single dye solutions at very low concentrations (Kaushik and Malik, 2009). For example, Baccar and collaborators (2011) tested *Trametes versicolor* using a single dye solution (Black Dycem TTO) at 150 mg/l in an air-pulsed reactor. The fungus was able to remove 86–89% of the tannery

dye over three batches but these promising results can be considered only poorly predictive of the actual fungal potential towards industrial effluents, which contain complex mixtures of dyes.

Obviously, these experiments give weak information about how a fungus could behave in a WWTP where it should compete with the autochthonous bacterial community, and at the same time efficiently degrade several dyes, even at high concentrations (Gao et al., 2010). Real polluted effluents represent an extreme environment that do not permit the optimal functionality of living organisms. Hence, their bioremediation can be carried out only selecting strains endowed by a strong degradation capability, stability and compatibility with the operative conditions set in industrial plants.

The ability to efficiently degrade dyes in controlled conditions does not surely indicate that the organism under study is potentially able to extensively act towards real wastewaters where the sterility cannot be maintained. Gao and collaborators (2008) observed a significant reduction of the fungal activity under non-sterile conditions: in axenic conditions, 89% of color reduction of a reactive dye was achieved by *Phanerochaete chrysosporium* within one day, whereas without sterility control, high decolorization yield (up to 80%) was observed after three days. In another experiment, a treatment with *T. versicolor* was set up in a 10 l bioreactor and exploited to degrade 150 mg/l of Grey Lanaset G, obtaining a 70% color reduction over three months (Blanquez et al., 2008). However, when the fungus was involved in the treatment of a real textile wastewater, only 40-60% of decolorization was reached. Moreover, under not sterile conditions, the process was less stable, lasting only 15 days (Blanquez et al., 2008).

1.3. Real extile wastewaters

1.3.1. Wastewater modification

Only few researches have dealt with real wastewaters to date and, in most cases, the obtained yields were lower than those expected (Hai et al., 2006; Blanquez et al., 2008). Fungal growth is strongly affected by the scarce nutrient resources present in the effluents, the high concentration of

detergents, heavy metals, salts and dyes, the extreme abiotic parameters as pH and temperature, and the presence of the autochthonous microflora.

Considering that textile industry daily produces large volumes of polluting effluents which should be treated fast and at low cost, some approaches can be applied only at lab scale: antibiotic addition, sterilization, etc. are not feasible strategies at industrial level. The final goal is to avoid as much as possible any effluent modification, which results in increased costs. In some cases, however, the modification of effluent parameters is strictly required.

Nutrient addition. Because of the low BOD of industrial wastewaters coming from dyeing processes, additional carbon sources are needed in order to sustain a stable and active fungal biomass over time. Faraco and collaborators (2008) using *Pleurotus ostreatus* to treat three simulated wastewaters observed that decolorization yields rose from 40% to 60% when additional nutrients were added. Similarly, since the effluent was poor of available carbon and nitrogen sources, *P. chrysosporium* was not able to work in absence of any nourishment (Faraco et al., 2008; Sangeeta et al., 2011). The addition of 5 g/l of glucose and 0.05 g/l of ammonium chloride was fundamental for *P. chrysosporium* to degrade several dyes (Radha et al., 2005).

As glucose is expensive, it may be substituted with carbohydrates-based wastes. For example, molasses have been assessed as alternative nutrients: a real textile wastewater was equally (60%) decolorized by *P. chrysosporium* in presence of 4 g/l of molasses and 1 g/l of glucose, suggesting that the more complex carbon source of molasses influenced the performance of the fungus (Pakshirajan and Kheria, 2012). A precise economical assessment is rarely performed but deeply recommended in order to define whether process yields improvement worth the use of high concentrations or expensive carbon sources. For example, the decolorization of a textile effluent (50%) rose to up to 80% and 60% in presence of 10 g/l of glucose and 4 g/l of molasses, respectively (Pakshirajan and Kheria, 2012). In that case, the feasibility of the process and the selection of the most appropriate nourishment should hence balance the economical impact of both the better-quality treated effluents and the carbon source supply.

pH lowering. The modification of this parameter is often crucial for the maintenance of a living and active organism. Industrial wastewaters show large pH fluctuations and are usually very alkaline (Vanhulle et al., 2008b). The optimal conditions for the fungal growth (pH 4-5) and for the maintenance of active enzymes (pH 3-5) should be taken as target points, but such acid values are not practically and economically sustainable by real plants (Kaushik and Malik, 2009). Neutral pH values can be instead considered a good compromise between the actual and the optimal working conditions. Actually many fungal treatments have been carried out at pH 7 with good results (Anastasi et al., 2010b; Park et al., 2011; Spina et al., 2012).

Moreover, even though contrasting data are available in literature, pH lowering is one of the possible strategies for the limitation of bacteria contamination (Gao et al., 2004; Gao et al., 2005). Bacteria are well known to produce proteases and compete for space and nutrients, disturbing or suppressing fungal activity (Libra et al., 2002; Hai et al., 2008) but in not sterile conditions, the development of the autochthonous microflora cannot be avoided. Yang and Yu (1996) acidified the culture medium of *P. chrysosporium* up to pH 3.1 in order to inhibit bacteria development. However, the fungal treatment was not able to remain stable in not-sterile conditions and the decolorization yield dropped down from 100% to 87% within the first 200 h.

Effluent dilution. Dilution of textile wastewater is a suitable solution to minimize the effect of high concentration of salts and other organic compounds on fungal growth. Diluted textile wastewaters were effectively degraded by *Trametes versicolor* (Libra et al., 2002), *Phlebia tramellosa* (Kirby et al., 2000), *Irpex lacteus* (Novotny et al., 2004), *Bjerkandera adusta* (Mohorcic et al., 2006) and *Aspegillus niger* (Assadi and Jahangiri, 2001).

COD and color removal may be favored by the contemporary wastewater dilution and nutrient addition. *P. chrysosporium* almost doubled its decolorization effectiveness when media containing glucose and other nutrients was substituted to water for the 1:1 dilution (Sangeeta et al., 2011). Similarly *Bjerkandera adusta* activity was strongly limited by the stringent conditions of a simulated reactive dye bath for cotton (pH 10, 70 g/l of salts and 5 g/l of dyes). On the contrary,

dilution made the degradation process possible: color of samples diluted at 1:3 and 1:5 was reduced of 45% and 72% respectively, indicating that the higher the recalcitrance, the more stringent the conditions for the growth and activity of the fungus become (Anastasi et al., 2010b). Besides, these yields of degradation were enhanced in presence of nutrients: 1:5 dilution and low N content medium was the only culture condition in which the fungus determined an almost complete degradation (91%) of even the most recalcitrant dyes, such as the reactive RY145 and RR195 and the direct DrY106 (Anastasi et al., 2010b).

However, considering that textile wastewaters are largely and continuously produced, and the hydraulic retention time inside WWTP should not exceed 1-2 days, this strategy seems to be unpractical at industrial scale.

1.3.2. Ecotoxicity evaluation

Powerful tools to assess the toxicity of untreated and treated samples are essential for a correct evaluation of the wastewater impact on the environment and their bioremediation process. Actually, chemical analyses do not allow to identify each chemical species and its potential harmful effects, because of the complex chemical nature of these wastewaters (Daniel et al., 2004; Latif and Licek, 2004; Sponza, 2006).

Moreover, bioassays consider the synergistic or antagonistic interactions occurring among chemicals (Daniel et al., 2004; Soupilas, 2008). Indeed, even a complete chemical characterization of a sample may be poorly predictive of all the side and combined effects that may happen in the real environment. Thus, the toxicological risk could be highly underestimated. Eventually, ecotoxicological analyses can take into account the whole effluent, evaluating also the effect of the chemico-physical parameters as pH, ionic strength, etc. In other words, ecotoxicity analyses can detected the total impact of the pollutants testing the industrial wastewater as a whole (EC Directive 2008/1 IPPC).

Acute toxicity tests are commonly used to detect the effect of pollutants on a certain organism after short exposure. On the contrary, chronic toxicity tests consider much longer time exposure and effects can be observed on the second generation, thus they are rarely applied in the monitoring of industrial wastewaters discharge because they do not allow rapid feedbacks (Sweet et al., 1997).

Different organisms have been standardized for ecotoxicity evaluation and the choice of the test organism should be guided by considering the characteristics of the wastewaters (i.e. color, turbidity) and the environment in which the wastewater is discharged. These organisms allow to observe different endpoints: luminescence inhibition (bacteria) photosynthesis inhibition (algae) vitality inhibition (or mortality) (phytoplankton, protozoa, crustaceans, rotifers, nematodes and fishes); growth inhibition (phytotoxicity towards mono- and dicotyledons). According to the kind of wastewater, the most sensitive organism can be *Pseudokirchneriella subcapitata* (Novotny et al, 2006; Tigini et al., 2011), *Cucumis sativus* or *Lepidium sativum* (Anastasi et al., 2011) or *Daphnia magna* (Tigini et al., 2011).

However, in order to have a more complete and correct evaluation of the wastewater ecotoxicity, a battery of ecotoxicological tests should be carried out, using organisms preferentially belonging to different trophic levels (Soupilas et al., 2008; Tigini et al., 2011). Nevertheless, the obtained data may be difficult to compare, since different toxicological principles and endpoints are used. Thus, results could be discordant (Latif and Licek, 2004; Novotny et al., 2006; Sponza, 2006; Soupilas et al., 2008). The use of indices allow to summing up the information obtained with different assays in a single value. Some simplified composite indices have been proposed, i.e. the Potential Ecotoxic Effect Probe (PEEP) assesses and compares the toxic potential of industrial effluents (Costan et al., 1993). However none of the indices has found general acceptance at the international level so they are still under development and implementation (Canna-Michaelidou and Christodoulidou, 2008).

1.4. Scale-up to large-volume reactors

Thanks to their capability to degrade xenobiotics, fungi could be used in large-scale wastewater systems, but they have rarely found an application in real plants to date. Unfortunately, also the most recent researches are still carried out in flasks (Saetang and Babel, 2010; Esmaeili and Kalantari, 2012; Senthilkumar et al., 2012). Only in few cases, bioreactors have been used to assess the behavior of fungal biomasses at larger working volumes (Anastasi et al., 2010b; Cerrone et al., 2011; Park et al., 2011; Rodarte-Morales et al., 2012).

The development of large-scale systems has been limited by the lack of appropriate reactors able to sustain fungal activity limiting undesired excessive mycelium growth, bacterial contamination and the washout of active extracellular enzymes and mediators produced by the fungus. Compared to bacteria, the development fermentation technologies suitable for fungi is delayed since very few is known about needs and responses to medium feeding, agitation/aeration rate and methodology, etc.

Reactors should be designed in order to avoid technical problems as system clogging, minimize the interferences on hypha growth and strengthen the fungal metabolism. Actually, in presence of an uncontrolled overgrowth of biomass, the liquid-mycelium interface diminishes and diffusion worsens, affecting oxygen and nutrient uptake by the fungus. These needs acquire major relevance considering that a close correlation have been pictured among technical stresses caused by fermentation operative parameters, fungal morphology and enzymatic activity which ultimately influence the decolorization capability of textile wastewaters. As an example, *Dichomitus squalens* produces laccases in a stirrer tank reactor and peroxidases in a bubble column reactor, and the enzymatic patterns were correlated with the pellet size and morphology (Babic and Pavko, 2012).

One of the main concerns is the reduction of the process yields obtained by the fungus, if scaled up in not optimal conditions. For example, *Funalia trogii* growth was scarcer in a bubble column than in flasks and its activity towards olive mill wastewaters in terms of color, COD and phenols content was delayed and worse (Cerrone et al., 2011). The degradation of a tannery dye was followed in flasks and in an air-pulsed reactor: the final results were similar but in reactor, *T. versicolor* needed more time to completely remove the color. After 24 h, flasks and the reactor showed 80% and 60%

of decolorization, respectively (Baccar et al., 2011). Obviously if the rate of degradation is too slow, it would be necessary to increase the hydraulic retention time during continuous processes.

Any reactor configuration has pros and cons that highly influences 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, loading factor, etc.). 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.

Some examples of reactor configurations that will be later discussed are presented in Figure 1.1.

Packed bed bioreactors can be set as trickling flow (with air in co- or counter-current flow) or submerged (up and down flow); in many cases the recycle of the liquid enhances the process efficiency. Being the flux rate of the liquid quite slow and the contact volume modest, huge sample volumes cannot be treated in a short time. Moreover, since the movement of the liquid avoids any turbulence, preferential flux paths and areas with different oxygenation may be formed. When excessive biomass growth also occurred, the low contact surface between liquid and solid phase becomes a limiting factor for the fungal treatment (Pocedic et al., 2009).

Many researches defined this kind of configuration optimal for fungal fermentation (Kaushik and Malik, 2009). A very stable system was set up for a *B. adusta* strain which remained active for a very long period (70 days and 10 cycles), extensively removing the color (average 84%) of a simulated and a real textile wastewater in non-sterile conditions (Anastasi et al., 2010b). Likewise, during the treatment of Reactive Black 5 by *Trametes pubescens* in a fixed bed reactor, the fungus stayed perfectly anchored to the support and mycelium was not released for more than 20 days treatment (Enayatzamir et al., 2009). The direct comparison of a stirrer tank reactor and a fixed bed one highlighted that the latter was the most suitable for the degradation of several pharmaceutical compounds by *P. chrysosporium* (Rodarte-Morales et al., 2012).

Membrane bioreactors use membranes to compartmentalize and protect the fungal biomass from the harsh environmental conditions, stabilizing it during time. This technology has many advantages, due to the free permeation of suspended solids and macro-colloids and the retention of high biomass concentration (Hai et al., 2006). For example, this system was able to sustain an active biomass of *Trametes* (*Coriolus*) *versicolor*, which was able to degrade almost 90% of a single dye solution with a hydraulic retention time of one day. Moreover, even working in not-sterile conditions, bacterial contamination was avoided (Hai et al., 2008). However, membrane fouling is a central problem and is mainly due to the deposit of broken hyphae and fungal compounds as polysaccharide, enzymes and reactive molecules onto the membrane (Shannon et al., 2008). Indeed, to overcome the external fouling by *T. versicolor,* several porous covering have been evaluated but in many cases fungal residues accumulated on the membrane as well as on the cover, i.e. nylon cloth (Hai et al., 2006). This phenomenon was minimized in presence of a mesh cage, where the layer of the fungus on the membrane did not perturb the effectiveness neither the endurance of the system. Actually, the fungus was stable throughout 50 days trial and decolorized a simulated textile wastewater up to 90%, causing even a partial mineralization (Hai et al., 2006). Therefore, the development of not-fouling membranes or additional technical solution is needed to define an economical and re-usable system.

Stirrer tank reactor is usually the best solution when high volumes of wastewater with suspended solids have to be treated since the mechanical agitation creates a good homogeneity of the liquid, enhancing the contact between pollutants, including particles, and the mycelium. *T. versicolor* was able to work continuously for more than three months towards single dyes solutions, with a final working volume of 4 l. Nevertheless, under not-sterile conditions, the competition with bacteria was strong and fungal activity suddenly dropped down after the first cycles (Borchert and Libra, 2001).

The optimization of the method performance may be achieved by in series process. For example, decontamination efficiency of a real dyeing effluent was strengthened by coupling two

mechanically-agitated reactors in which *P. chrysosporium* was able to remove 54% and 79% of the color and COD, respectively (Park et al., 2011).

Many factors have to be controlled (i.e. agitation rate, size and shape of impellers, etc.) in order to avoid any mechanical stress on the fungal biomass. Actually, stirring rate influences the homogeneity of the liquid as well the aeration, allowing to correlate the mechanical agitation provided by impellers to the oxygen bioavailability. At high speed (above 150 rpm), the system achieved its optimal running conditions faster, reducing the time needed to get the maximal dissolved oxygen and oxygen uptake. It was possible to associate these data with the fungal development, evidencing a consistent anticipation of the exponential growth phase in comparison with low (50-100 rpm) agitation rate (Singh and Dikshit, 2011). However, the agitation rate should be carefully increased in order to avoid mechanical and hydrodynamic stresses. For example, when a stirrer tank reactor was run at 250 rpm, the fungus decolorization capability was lessened in comparison to lower stirring rate, probably due to the observed morphological changes (Singh and Dikshit, 2011).

Moreover, both the size and the geometry of the impellers are important. For example, 7 different propellers have been evaluated for their capability to maintain a proper carrier suspension in minimum rotational speed, influencing the mechanical constrains. Pitched blade turbines (Mixel TTP, 45° oriented) and elephant ear impellers achieved the required suspension and homogenization performances, while minimizing mechanical stresses (Collignon et al., 2010).

Bubble column bioreactor is technically simpler than a stirrer tank reactor, since it is not mechanically agitated and air bubbles are responsible of the liquid mixing and the consequent mass transfer on the gas-liquid surface. Aeration inject has to be controlled: reactors performances usually decrease with the increase of aeration speed. Indeed, as a consequence of high agitation rate, bubbles enlarge and the larger bubbles are formed, the lower surface interaction is established between liquid and gas phase, reducing mass transfer rate (Kartarci et al., 2005). In addition,

avoiding any moving part as motors and stirrers, less energy is required and the maintenance costs are lower, representing a great advantage at industrial scale.

Trametes versicolor in an air-pulsed reactor was able to decolorize more than 85% of a single dye solution over three repeated 4-days cycles (Baccar et al., 2011). In comparison with free pellets of *Phoma* sp. in flasks, immobilized biomass in bubble coloumn achieved better decolorization yields of a direct dye. This observation may find its basis on the more controlled aeration in reactor, which enhanced the availability of oxygen and consequentially improved fungal effectiveness (Junghanns et al., 2012). Oxygen transfer appears to be the crucial feature of bubbles columns, whose optimization leads to more active fungi. Thereby, this was indicated as the reason of the monitored differences between lab-scale and pilot-scale bubble columns: in the latter, *T. versicolor* grew faster, showing a higher glucose consuming rate and the maximal decolorization yield (90%) of Grey Lanaset G dye was get faster too (Blanquez et al., 2008).

1.5. Immobilization of fungal biomass

As discussed before, the overgrowth of the fungus as dispersed mycelium has strong repercussions on the fungal treatment effectiveness and endurance, mainly because of reactor clogging and limited mass transfer. With the attempt to minimize this phenomenon, the biomass may be immobilized on supports. This approach could make the process more compatible at industrial level, limiting uncontrolled mycelium development, allowing the re-use of the fungal biomass and reducing time and resources for the separation of the biomasses (Rodriguez-Couto, 2009; Gao et al., 2010). Obviously, a reusable system, which needs few downstream controls, positively influences the final economical balance of the process.

Immobilized fungi are less affected by collisions and share damages due to the mechanical agitation that may destabilize dispersed mycelium. Supports primarily give physical protection to the fungus. Moreover, immobilized biomass usually shows higher resilience to environmental perturbation associated to the chemical and physical conditions of industrial wastewaters. Extreme and varying

pH values, high organic load, etc., have a lower effect on colonized carriers than free pellets (Shin et al., 2002). As a consequence, in some cases, supported mycelium showed a higher enzymatic production compared with free pellets (Gao et al., 2010; Spina et al., 2012).

Fungal immobilization can be obtained by entrapment or attachment. In the first case, the fungus colonizes the pores of the carrier growing also inside it, whereas in the second case, it only adheres and attaches to the superficial surface (Rodriguez-Couto, 2009).

The crucial point is the selection of appropriate supports in terms of shape and composition. Taking in mind the unique habit of fungi (exploring hyphae, conidia, no biofilm formation, etc.), they generally have a low adaptation capability onto solutions specifically designed for other microorganisms, i.e. bacteria.

The three-dimensional structure deeply affects the development of an active and stable biomass: high porous supports allow a better diffusion of nutrients and oxygen (Pocedic et al., 2009). Rodriguez-Couto (2012) observed that the hydrophobic surface enhanced the adhesion of the mycelium to the supports. The chemical and physical properties of the supports influence the liquid phase distribution, the liquid hold-up in bed and the time diffusion with direct consequences on the mycelium. In fact, part of the medium has to be entrapped inside the colonized carrier in order to sustain biomass growth and enzymatic production. Polyether macro-reticulate foam, cosmetic luffa sponge and polyamide kitchen scourers guaranteed an optimal surface of interaction for the *I. lacteus* growth (Pocedic et al., 2009).

The specific environmental stresses to which supports are exposed have to be carefully considered. Carriers should not interact with textile effluents, maintaining their structural integrity even in presence of high concentration of salts and aromatic compounds and both in acid and alkaline pH. Hence, the use of beads of resins and polymers is strictly limited by their stability in extreme chemical and physical conditions (Wang and Hu, 2007; Pazarlioglu et al., 2010). For example, although alginate beads have been successfully used for dye degradation in controlled conditions over more than 40 days (Dominguez et al., 2005), they are strongly influenced by pH. The strength

of the particles may be modulated by alginate concentration (Park et al., 2006; Pazarlioglu et al., 2010), but they are unstable in phosphate and citrate at alkaline pH values (Arica et al., 2001).

The selection of the supports should be thus focused on inert materials avoiding the adsorption of wastewater compounds as well as the release of carrier particles (Gao et al., 2010). Polyurethane foam (PUF) and stainless scourers fit this general requirement and have been successfully used to treat model single dye solutions (Hai et al., 2006; Enayatzamir et al., 2009; Pocedic et al., 2009; Novotny et al., 2011) and real samples, being imperturbable to real environmental conditions of textile wastewaters (Anastasi et al., 2010b; Novonty et al., 2011; Park et al., 2011; Spina et al., 2012). Thereby, some concerns rise about the use of scourers in moving-bed reactors. In fact, due to their structure and weight, they may cause scraping damages of the inner surface of a tank and the movement maintenance requires elevate energy consumption.

Besides, lignin-derivates wastes are considered interesting tools for fungal immobilization. They simulate the physiological environment where fungi live, stimulating secondary metabolism and providing additional nutrients (Li and Jia, 2008; Rodriguez-Couto, 2009). However, they may also release aromatic compounds, worsening organic polluting load of wastewaters (Forss and Welander, 2009).

The specific advantage of both inert materials and agro-wastes has induced many researchers to deepen and compare their feasibility as fungal supports for textile wastewater treatment. Pine-wood and PUF capability to sustain an active mycelium have been assessed finding that peroxidases were induced by the presence of a lignin-derivate matrix. However, this does not always reflect a better reactivity of the fungus, since a clear correlation between degradation and secreted oxidative enzymes was not detected (Susla et al., 2007). In fact, better decolorization of Reactive Orange 16 and Remazol Brilliant Blue were observed in presence of colonized PUF, suggesting the probable involvement of diverse peroxidase isoforms or other enzymes (Novotny et al., 2004; Susla et al., 2007).

1.6. Fungal treatment integration in existing WWTPs

Due to the complex and heterogeneous composition of textile wastewaters, a unique way of treatment is unrealistic. WWTPs usually combine several classic treatments based on both biological (activated sludge), physical and chemical techniques (Robinson et al., 2011), but there is still room for improvements along the process line. Indeed activated sludge is the most common biological treatment used in plant but they have significant limitations, in particular towards the effluent color (Novotny et al., 2011).

Up to date, biological techniques are usually coupled with chemical ones (Zhang and Yu, 2000; Robinson et al., 2001). For example, *P. chrysosporium* gave an important contribution to the color (79%) and COD (54%) removal from a dyeing wastewater, but process efficiency was improved by the following chemical coagulation. Thanks to the combined approach, the values rose to 96% and 73%, respectively (Park et al., 2011). A combined ozonation-fungal process was also investigated, proving to be more efficient than the two single processes (Vanhulle et al., 2008b). The effluent toxicity was reduced of 10% by ozonation, 35% by *Pycnoporus sanguineus* and 70% by the integration of the two methods.

An alternative solution can be given by a combined biological process, based on fungi and bacteria synergistically cooperating to achieve a complete wastewater decontamination. Indeed, strong color and COD reduction are generally imputable to fungi and bacteria, respectively. Hence, both fungi and bacteria can be used to set up an integrated system, complementary working and completing each other (Novotny et al., 2011; Anastasi et al., 2012; Spina et al., 2012). In overall, reducing the organic content, together fungi and bacteria can mediate a significant detoxification of textile wastewaters. Indeed, it was assumed that bacterial activity may be enhanced against an already-treated wastewater, because fungi degraded recalcitrant molecules that can be instead toxic for bacteria thus limiting their functionality (Spina et al., 2012).

Besides, the choice of the process scheme should take into consideration several factors balancing economical sustainability and process yields in order to get the best from each method. Chemicals

dosage, sludge production and the presence of inhibitory or non-biodegradable substances have to be considered to plan the WWTP profile (Hai et al., 2006).

Fungal treatment inserts in this general picture and as well the correct position inside the plant have to be determined. In particular, since fungi suffer bacterial competition (Blanquez et al., 2008; Novotny et al., 2011), their integration after active sludge can be risky because the microbial load is high even in presence of ultrafiltration steps. Moreover, fungi have demonstrated to be mostly active towards the color (Novotny et al., 2011; Spina et al., 2012), being non-sense their exploitation towards almost limpid waters. On the other hand, since COD reduction by fungi is not a certainty (it depends on water quality, chemical load, effluent modifications, etc.), other technologies in the next phase are needed, in order to lower COD values and to continue the toxicity reduction.

1.7. Conclusion

Fungi can be considered powerful tools to be applied in textile wastewater treatment. A whole-cell approach still seems to be the most feasible solution: primary due to the stringent and extreme chemical and physical conditions, enzymes can be strongly inactivated by real effluents. The strength and the robustness of selected fungal strains are thus required and highly recommended.

In order to develop a fungal treatment active as long as possible and applicable in real WWTPs, some features have to be taken into consideration and carefully investigated. Fungi must be enabled to remain active in the hursh conditions of real wastewaters mitigating the extreme physico-chemical conditions and/or bacteria competition and enhancing their potential degradation tools. The aspecific oxidation machinery expressed by fungi makes them among the most promising green biocatalysts involved in industrial wastewater treatment.

Several strategies and new technologies have been developed. Thanks to this continuous evolution and the constant improvements, research is now able to offer different solutions to sustain fungal growth and activity at large scale, which, together the most proper reactor configuration and the

choice of the suitable supports, will allow the potential of fungal treatments to be exploited even at industrial scale.

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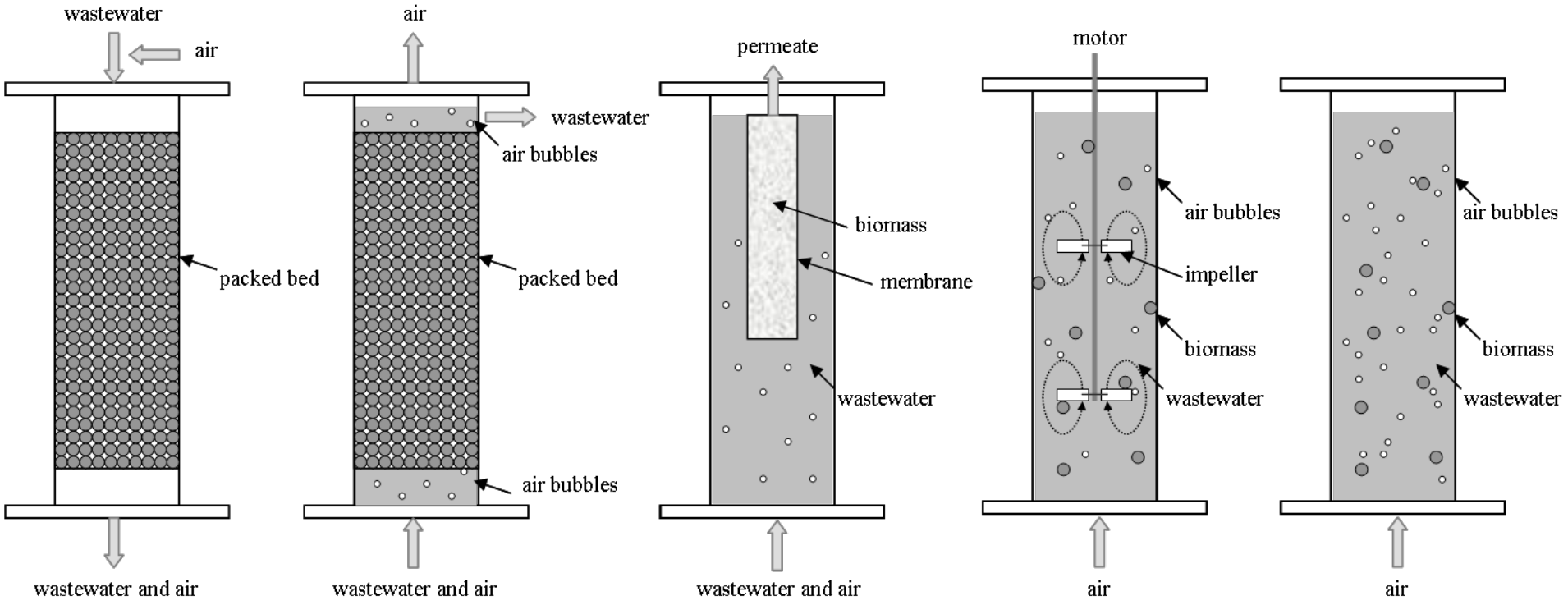
Figure 1.1: Fixed bed reactor (trickled or submerged), membrane reactor, stirrer tank reactor and bubble column.



Tricled bed rector (co-current flow)

Submerged bed reactor (up flow)

Membrane reactor

Stirrer tank reactor

Bubble column