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Review

Utilization of fungi for biotreatment of raw wastewaters

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Fungal biomasses are capable of treating metal-contaminated effluents with efficiencies several orders of magnitude superior to activated carbon (F-400) or the industrial resin Dowex-50. Additionally, fungal biomasses are susceptible to engineering improvements and regeneration of their capabilities. With regard to organic pollutants, excessive nutrients and dyes, fungi can remove them from wastewaters, leading to a decrease in their toxicities. However, the detoxification rates seem to be dependent on media and culture conditions. The postreatement by anaerobic bioprocesses of effluents that have been pretreated with fungi can lead to higher biogas than the original effluents. In addition to the degradation of organic pollutants, fungi produce added-value products such as enzymes (LiP, MnP, Lacc, amylase, etc.) and single-cell protein (SCP). Most research on fungal capacities to purify polluted effluents has been performed on a laboratory scale, hence there is a need to extend such research to pilot scale and to apply it to industrial processes.

Key words: Wastewaters, effluents, fungi, biodegradation, biosorption, decolourisation, value-added treatment.

INTRODUCTION

Fungi are recognized for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints (Lilly and Barnett, 1951; Cochrane, 1958). For example, *Aspergillus niger* is the prototypical fungus for the production of citric acid (Clark, 1962; Lal, 1980; Grewal and Kalra, 1995), homologous proteins (esp. enzymes) and heterologous proteins (Archer et al., 1994; Prasertsan et al., 1997; Radzio and Kuck, 1997; Wongwicharn et al., 1999; Xu et al., 2000). Moreover, *Phanerochaete chrysosporium* is the model of white-rot fungi for the production of peroxidases (Bumpus et al., 1985; Rodriguez et al., 1999). Beyond the production of such relevant metabolites, fungi have been attracting a growing interest for the biotreatment (removal or destruction) of wastewater ingredients such as metals, inorganic nutrients and organic compounds (Akthar and Mohan, 1986; Field et al., 1993; Feijoo and Lema, 1995; Palma et al., 1999; Coulibaly, 2002).

The focus of this review therefore concerns the use of fungi to remove or degrade various wastewater constituents. Some instances of synthetic wastewaters are reported, but only the contributions of fungal biomass in the biological treatment of raw wastewater are discussed in some length.

DOMESTIC SEWAGE

Domestic sewage contains carbon and nutrient sources that can be removed by fungal biomass. In an early

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Abbreviations; SCP: single-cell protein, LiP: lignin peroxidase, MnP: manganese peroxidase, MIP: manganese independent peroxidase, Lacc: laccase, COD: chemical oxygen demand, HTL: heat treatment liquor, BOD: biochemical oxygen demand, OMW: olive mill wastewaters, WWTP: wastewater treatment plant.

investigation, Thanh and Simard (1973) demonstrated the capacities of seventeen fungal biomasses to remove phosphates (84.1%), ammonia (73.3%), total nitrogen (68.1%) and chemical oxygen demand (COD) (39.3%). They obtained fungal growth on this effluent with an accumulation of biomass (451.2 mg l^{-1}) that contained protein (47% g g^{-1}). There was variability in fungal capacities as to the removal of pollutants (see Table 1). In fact, *Trichothecium roseum* was the best in phosphate removal (97.5%), whilst *Epicoccum nigrum*, *Geotrichum candidum* and *Trichoderma sp*. were the best in the removal of ammonia (84%), total nitrogen (86.8%) and COD (72.3%), respectively. Concerning cell-protein production, *Paecilomyces carneus* had the highest ratio of protein to biomass (92.5%). However, this fungus did not grow very well on domestic sewage. In our laboratory, domestic wastewater pretreatment by a strain of *A. niger* has been investigated under transient conditions. This fungal biomass removed about 72% of COD and 65% of protein (Coulibaly, 2002). Despite the differences between the bioprocess investigated in these two studies, COD and protein removal rates are in the same order. The overall feasibility of domestic wastewater treatment under sewer-simulating conditions has been explored recently both experimentally and by simulation (Coulibaly, 2002; Coulibaly et al., 2002; Coulibaly and Agathos, 2003). The heat treatment liquor (HTL) of an activated sludge was decolourised by *Coriolus hirsutus* (Fujita et al., 2000). This fungal strain exhibited a strong ability to decolourise HTL (70%) with an accumulation of manganese independent peroxidase (MIP) and manganese peroxidase (MnP). Optimising the culture medium by adding nitrogen and carbon sources and improving the biomass quality resulted in increased colour removal capacity by *C. hirsutus* (Kumar et al., 1998; Miyata et al., 2000; Fujita et al., 2000). Although fungal applications have shown good capacities on sewage treatment, they are still underutilised in practice. This could be explained, in part, by a widespread *a priori* assumption that fungal strains do not perform as well as bacteria.

AGROINDUSTRIAL EFFLUENTS

Industries of olive oil, tapioca starch, distillery (molasses), cotton bleaching, pulp and paper processing produce several billion litres of coloured, often toxic and harmful wastewaters over the world annually. Those effluents have strong concentrations of COD (10-200 g l^{-1}), phenol and its derivatives (0.5-8 g l^{-1}) and often contain proteins, cyanides, chlorinated lignin compounds and dyes (Borja et al., 1992, 1997; Nieto et al., 1992; Bengtsson and Triet, 1994; Garcia et al., 1997; Jimenez and Borja, 1997; Yesilada et al., 1998; Kahmark and Unwin, 1999). The large amount of lignin derivatives of these effluents is responsible of their dark-brown colour (Calvo et al., 1995). The phenolic compounds of such wastewaters exert some bactericidal effects on wastewater treatment plant (WWTP) microorganisms (Borja et al., 1996; Fang and Chan, 1997; Vassilev et al., 1997; Sayadi et al., 2000). Fungal pretreatment (Table 2) of these effluents under aerobic conditions makes it possible to obtain phenol reduction (51-100%), good decolourisation (31-100%), biochemical oxygen demand (BOD) reduction up to 85.4%, and enzyme production (protease, Lacc (EC 1.10.3.2); LiP (EC 1.11.1.14), MnP (EC 1.11.1.13), amylase, etc.) (Vinciguerra et al., 1995; Yesilada et al., 1995; Garcia et al., 1997, 2000; D'Annibale et al., 1998; Setti et al., 1998; Gharsallah et al., 1999; Robles et al., 2000; Kissi et al., 2001). Amendment of olive mill wastewater (OMW) composition (addition of co-substrate, nutrients, salts) influences the removal of COD, phenols and colours (Yesilada et al., 1998). In fact, Garcia et al. (2000) noted that *G. candidum* removed COD but did not degrade phenols. However, by optimising OMW composition (COD:N:S = 100:5:2) for *G. candidum* growth, Assas et al. (2000) obtained a complete degradation of phenols and 70% decolourisation. Miranda et al. (1996) maximized colour removal from molasses wastewaters (up to 69%) with *A. niger*, after the amendment of the culture medium with co-substrate and mineral nutrients ($MgSO_4$, KH_2PO_4 and NH_4NO_3). Some of the consequences of OMW pretreatment by fungi are the 23- to 30-fold higher increases in biogas production and the fertilizing effect on plants (*Trifolium repens*) compared to non-pretreated effluent (Borja et al., 1993, 1995 a,b,c; Jimenez and Borja, 1997; Vassilev et al., 1998).

The influence of co-substrate (see Table 3) upon paper and pulp industrial wastewater treatment, detoxification and decolourisation rates has also been observed with *Ceriporiopsis subvermispora*, *P. chrysosporium*, *Trametes versicolor*, *Rhizopus oryzae* and *Rhizomucor pusillus* (Manzanares et al., 1995; van Driessel and Christov, 2002; Nagarathnamma and Bajpai, 1999; Nagarathnamma et al., 1999). The mechanisms of decolourisation of agroindustrial effluents by fungi are reported to include biosorption and/or biodegradation (Ohmomo, 1988; Sayadi and Ellouz, 1995; Soares and Duran, 1998; Christov et al., 1999; Nagarathnamma et al., 1999). Some "mycoreactors" such as rotating biological contactor (MYCOR), trickling filter reactor (MYCOPOR) and continuous column reactor have been developed to decolourise pulp and paper wastewaters (Eaton et al., 1982; Messner et al., 1990; Bajpai et al., 1993). These reactors were able to run over several weeks by maintaining their colour removal rates. Ligninolytic enzymes are also involved in the degradation of organic compounds, including dyes (see below), within these effluents (Chivukula et al., 1995). The enzymatic oxidation mechanism of those pollutants has been well discussed elsewhere and is not the aim of this contribution (Young and Yu, 1997; Mester and Tien,

Table 1. Examples of fungi used to treat domestic sewage, starch processing and metal bearing effluents. Optimal culture condition and the effect of fungal pretreatment are reported.

Effluents	**Fungi**	**Treatment**		**References**
		Reactor and medium handling	Parameters	
Domestic sewage	*Penicillium citricum, Steganosporium piriforme, Arthrinium arundis, Fusarium oxysporum, Cladosporium herbarum, Cladosporium cladosporioides, Scopulariopsis brevicaulis, Mucor hiemalis, Trichothecium roseum, Epicoccum nigrum, Helminthosporium sativum, Ulocladium atrum, Geotricum candidum, Trichocladium asperum, Paecilomyces carneus, Trichoderma sp., Chrysosporium pannorum*	Shake-flask	COD (72.3%); Phosphates (97.5%); N-total (86.8%); Dry matter (684 mg l^{-1}); Protein content (205 mgl^{-1})	Thanh and Simard (1973)
	Aspergillus niger	Stirred tanks reactor in series	COD (72%); N-total (65.4%)	Coulibaly (2002)
	Coriolus hirsutus	Continuous immobilized bioreactor; addition (nutrient (NH_4 (100 mg l^{-1}), NO_3 (100 mg l^{-1}); $MnSO_4$); co-substrate (glucose, 0.5%)	Decolorization (80%, 2 d); MnP (60 U l^{-1}); MIP (40 U l^{-1})	Miyata et al. (2000)
Starch processing effluent	*A. oryzae; Rhizopus arrhizus; Trichoderma viride; T. reesei; G. candidum; A. terreus; R. oligosporus*	Shake-flask, air lift bioreactor (45 l); addition of nutriment $(NH_4)_2SO_4$; Urea; NH_4NO_3; $NaNO_3$; K_2HPO_4; KH_2PO_4)	TOC (44-88%); SS (95%); starch hydrolysis (53-100%); biomass (2-5.6 g l^{-1}); protein (48.8% of biomass weight); COD (97.8%); glucoamylase (3.94 U ml^{-1})	Jin et al. (1999abc; 2001)
	A. niger; A. oryzae	Shake-flask	COD (90%); biomass and amylase production	Fujita et al. (1993); Murado et al. (1993)
Metal bearing effluent	*A. niger, P. simplicissimum, Geotrichum sp., Fusarium verticillioides, Rhizoctonia solani, Aquathanatephorus pendulus;*	Shake-flask, presence of co-ions, biomass (produced)	*A. niger* (Cu (91%); Zn (70%))	Price et al. (2001); Gomes et al. (1998, 1999); Gomes and Linardi (1996); Karavaiko et al. (1996)
	A. niger, A. flavus, A. fumigatus; R. Arrhizus; A. terrus	Shake-flask; presence of co-ions, biomass (industrial waste, produced)	Metal removal (82-100%)	Balakrishnan et al. (1994); Niyogi et al.(1998)
	Mucor meihi	Shake-flask; biomass (industrial waste), dilution (1-20)	Sorption (0.7-1.15 mmol g^{-1})	Tobin and Roux (1998)
	A. niger	Shake-flask; presence of co-ions, biomass (produced)	Metal removal (75%)	Akthar and Mohan (1995)

Table 2. Examples of fungi used to treat distillery wastewaters. Optimal culture condition and the effect of fungal pretreatment are reported.

Effluents	**Fungi**	**Treatment**		**References**
		Reactor and medium handling	Parameters	
Distillery Wastewaters	*A. awamori* var. *kawachi*	Shake-flask; Jar-fermentor (30 l)	Specific resistance of culture broth (97.5% decrease); BOD (56%); TOC (72%); Phosphates (80%) protein in mycelium (40%) Increased TOC removal of anaerobic pretreated effluent	Kida et al. (1995)
	A. niger; A. awamori	Shake-flask; continuous bubble reactor; co-substrate (sucrose, fructose, glucose); $MgSO_4$ (1 gl^{-1}); KH_2PO_4 (0.5 gl^{-1}); NH_4NO_3 (1.8 gl^{-1}); peptone (5%); rice (3%)	OMW (decolorization (69%, 3-4 d); COD (78%)) Thin stillage (protease (200 U ml^{-1}); biomass (30 g l^{-1}))	Miranda et al. (1996); Yang and Lin (1998)
	P. chrysosporium; G. candidum; C. versicolor; Mycelia sterilia	Dilution (50%)	Decolorization (53%, 10 d); growth rates inhibition below 50% of dilution; decoloration of melanoidins (80%) by *P. chrysosporiumJAG-40*	FitzGibbon et al. (1998)
	G. candidum	Shake-flask; Jar-fermentor (7 l); Polyurethane-foam; immobilization; co-substrate (glucose, 0.5-1%)	Decolorization (80%), peroxidase accumulation	Kim and Shoda (1999)
	Trametes versicolor	Shake-flask; inoculum size, sucrose addition (0.3%); KH_2PO_4 (0.5 gl^{-1});	Decolorization (82%); COD (77%); NH_4^+ (36%)	Benito et al. (1997)
	C. hirsutus	Shake-flask; continuous immobilized polyurethane-foam reactor; $MnSO_4$; co-substrate (glucose, ethanol)	Decolorization (76%, 2 d); TOC (45%);	Miyata et al. (2000)
	Flavodon flavus; P. decumbens	Shake-flask; aeration; co-substrates (sucrose, glucose, mannose, mannitol, xylose, arabinose, fructose, glycerol) tested at 10%	Decolorization (80%); MnP(400 U l^{-1}); Lacc (550 nkat l^{-1}); increase of anaerobic digestion rate of aerobic pretreated effluent by P. decumbens	Raghukumar and Rivonkar (2001); Jimenez and Borja (1997)
	Ceriporopsis subvermispora	Shake-flask; co-substrat (glucose, 0.1%; sucrose; lactose; microcrystalline cellulose; carboxymethyl cellulose; xylose; starch; athyl alcohol; bagasse pith; cheese whey; prehydrolysate liquor; molasses)	Color (90%); COD (45%); lignin (62%); AOX (32%); EOX (36%)	Nagarathnamma et al. (1999)

Table 3. Examples of fungi used to treat wood processing wastewaters. Optimal culture condition and the effect of fungal pretreatment are reported.

Effluents	Fungi	Treatment		References
		Reactor and medium handling	Parameters	
Wood processing wastewaters	*Sporotichum pulverulentum* (*P. chrysosporium*)	Batch reactor (25 m^3); continuous laboratory fermentor (10 l)	Biomass (5.7 g l^{-1}); Protein (42%); Protein productivity (132 mg l^{-1}); cellulase (0.2 U ml^{-1}); Suspended particles (88%); BOD 73%); COD 52%); feedstuff (rat, pigs and sheep)	Ek and Eriksson (1980); Thomke and Rundgreen (1980)
	A. foetidus	Shake-flask; dilution (10%)	Decolorization (90%, 2 d)	Sumathi and Phatak (1999)
	C. versicolor; P. chrysosporium; Pleurotus ostreatus; polyporus versicolor	Shake-flask; immobilization in beads of Ca-alginate gel; dilution (16.7%); co-substrates (sucrose 0.5%, xylose, glucose, glycerol, ethanol)	Decolorization of suspended biomass (60%, 6 d); decolorization of immobilized biomass (80%, 3 d)	Livernoche et al (1980); Marwaha et al. (1998)
	P. chrysosporium; Phanerochaete flavido-alba	Shake-flask; co-substrate (glucose); Mn (0.3 mg l^{-1}); culture age	Decolorization (88%), LiP (450 nmol min^{-1} ml^{-1}); MnP (800 nmol min^{-1} ml^{-1})	Perez et al. (1997)
	P. chrysosporium; Ganoderma australe; Coriolopsis gallica ; Paecilomyces variotii	Shake-flask	*P. chrysosporium* (decolorization, 50%, 7 d; lignin pyrolisis compounds, 57% reduction;); *G. australe* (decolorization, 50%, 7 d; lignin derivated compounds 48% increased); *C. gallica* (decolorization, 48%, 7 d; lignin-derivated compounds 77% reduction); *P. variotii* (decolorization, 85%, 7 d; lignin-derivated compounds 78% reduction)	Calvo et al. (1995 a b)
	T. versicolor	Shake-flask; continuous feeding bioreactor; culture age; dilution 30%); SO_4Mn (23 mg l^{-1}); co-substrate (glucose, 0.3%; sucrose; starch; ethanol, carboxymethyl-cellulose; pulp and bagasse pith)	Decolorization (90 %, 9 d) Lacc (700 U l^{-1}, 10 d); MnP (25 U l^{-1}, 7 d); phenols (90%); COD (69%)	Manzanares et al. (1995); Mehna et al. (1995)
	Sordaria fimicola; Halosarpheia ratnagiriensis	Shake-flask; pH (4.5; 8.2)	*S. fimicola* (decolorization, 55%, Lacc (100 nkatal ml^{-1})); *H. ratnagiriensis* (decolorization, 85%; Lacc (100 nkatal ml^{-1}))	Raghukumar et al. (1996)
	Ceriporopsis subvermispora; R. oryzae	Shake-flask; co-substrat (glucose, 0.1%; sucrose; lactose; microcrystalline cellulose; carboxymethyl cellulose; xylose; starch; athyl alcool; bagasse pith; cheese whey; prehydrolysate liquor; molasses)	Color (90%); COD (45%); lignin (62%); AOX (32%); EOX (36%)	Nagarathnamma et al. (1999); Nagarathnamma and Bajpai (1999)

2000). Beneficial effects of the fungal pretreatment of pulp mill effluent upon its subsequent anaerobic digestion have been reported (Feijoo et al., 1995). Anaerobic digestion of Kraft pulp mill effluent pretreated by *P. chrysosporium* gave increased degradation of high molecular weight compounds (79%) according to these authors. Also, an important decolourisation (79%) was also observed, that was correlated with MnP accumulation.

With regard to other agroindustrial wastewaters that are relatively non toxic (e.g. dilute lignocellulosics, starch, rice and mussels processing, sauce production, etc.) (see Table 1), fungal growth on them has been reported to produce single-cell protein (SPC), enzymes, chitosan, amylolytic preparations and a good reduction of COD (up to 97.8%) (Morimura et al., 1992, 1994 a,b; Murado et al., 1993; Kida et al, 1995; Yang and Lin, 1998; Yokoi et al., 1998; Jin et al., 1998, 1999, 2001).

DYED EFFLUENTS

The effluents of pharmaceutical industries, dyeing, printing, photographs, textile and cosmetics contain dyes (McMullan et al, 2001). For example, over 7 X 10^7 tons dyes are produced annually worldwide, of which about 10% are lost in industrial effluent (Vaidya and Datye, 1982). Wastewaters from textile industries are a complex mixture of many polluting substances such as organochlorine-based pesticides, heavy metals, pigments and dyes. Their compositions have been discussed in detail by O'Neill et al. (1999). The majority of these dyes are slowly removed by the WWTP, because of their toxicities to indigenous microorganisms. Dye removal from wastewaters by established WWTP processes are expensive and need careful application (Vandevivere et al., 1998; Robinson et al., 2001). Furthermore, following anaerobic digestion, nitrogen-containing dyes are transformed into aromatic amines that are more toxic and mutagenic than the parent molecules (Shaul et al., 1985; Chung and Stevens, 1993; Ganesh et al., 1994). To overcome these difficulties, fungi are being investigated for their potential to decolourise effluents. Among them, the most widely studied are the white-rot fungi *P. chrysosporium* (a model, primarily laboratory organism) and *T. versicolor* (a promising organism for industrial applications).

Nowadays other fungi have also shown some capacities to remove dyes from industrial effluents. Dyes are removed by fungi by biosorption (Contato and Corso, 1996; Tatarko and Bumpus, 1998; Payman et al., 1998; Zheng et al., 1999; Fu and Viraraghavan, 2000), biodegradation (Nigam et al., 1995; Conneely et al., 1999) and enzymatic mineralisation (LiP, MnP, manganese independent peroxidase (MIP), Lacc) (Young and Yu, 1997; Ferreira et al., 2000; Ollikka et al., 1998; Podgornik et al., 1999; Wong and Yu, 1999; Zheng et al., 1999; Pointing and Vrijmoed, 2000; Wesenberg et al., 2003). However, one or more of these mechanisms could be involved in colour removal, depending on the fungus used. Other fungal biomasses applied to the decolourisation of raw textile effluents include *Botrytis cinerea*, *Endothiella aggregata*, *Geotrichum fici, R. oryzae*, *Tremella fuciformis*, *Xeromyces bisporus*, *Hirschioporus larincinus*, *Inonotus hispidus*, *Phebia tremellosa* and *C. versicolor* (Banat et al., 1996; Kirby, 2000; Polman and Breckenridge, 1996). It is reported that raw effluents can only partially be decolourised upon fungal treatment (maximum of 49-80% but often much less). For example, a complex mixture of real textile effluents containing many reactive dyes could be decolourised upon partial dilution by using the agaric white-rot fungus *Clitocybula dusenii* (Wesenberg et al., 2002). The weak decolourisation of these effluents by complete cultures could be explained by the influences of temperature, pH, salts, inhibitory molecules (sulphur compounds, surfactants, heavy metals, bleaching chemicals), carbon and nutrients within these solutions (Chao and Lee, 1994; Jacob et al., 1998; Swamy and Ramsay, 1999; Mester and Tien, 2000). Concerning enzymatic (Lacc, LiP, MnP) degradations, these reactions are quite complicated, involving numerous low molecular weight cofactors that serve as redox mediators (Reyes et al., 1999; Wesenberg et al., 2003). These cofactors, in addition to the enzymes themselves, influence fungal colour removal rates.

METAL CONTAINING EFFLUENTS

Metallurgical industries, mining, surfaces cleaning, waste incinerators produce large wastewater polluted by metals. Dissolved metals escaping into the environment pose a serious health hazard. Because they accumulate in living tissues throughout the food chain, which has human at its top. There is a need to remove heavy metals before they enter the complex ecosystem. Physicochemical treatments evolved in very diluted water-containing metals (precipitation, electrochemical, flocculation, coagulation, ion exchange) are expensive. Utilization of biomasses in general (Volesky, 1994; Veglio and Beolchini, 1997; Kratochvil and Volesky, 1998; McKay et al., 1999; Gupta et al., 2000) and particularly that of fungi are considered to be best alternatives for those waters purification (Kapoor and Viraraghavan, 1995; Modak and Natarajan, 1995; Sag et al., 1998; Volesky and Holan, 1995; Atkinson et al., 1998; Kratochvil and Volesky, 1998; Mogollon et al., 1998; Savvaidis, 1998; Tobin and Roux, 1998). Indeed, the purification of the water-containing metals by fungal biomass is cheaper and it presents the following advantages: (i) production of residual small volume; (ii) possibility of valorisation of fungal waste biomasses from industrial fermentations; (iii) fast removal and (iv) easy installation of the process.

Fungal biomasses walls are composed of macromolecules (chitin, chitosan, glucan, lipid, phospholipides), which contain carboxyl groups (R-COOH), amino groups (R_2NH, $R-NH_2$), phosphates, lipids, melanin, sulphates ($R-OSO_3^-$) and hydroxides (OH^-) (Caesartonthat et al., 1995; Kapoor and Viraraghavan, 1998 a,b; Fogarty and Tobin, 1996; Kapoor et al., 1999). Those functional groups are metals sorption sites (Tsezos and Volesky, 1982; Mullen et al., 1992; Guibal et al., 1995; Gardea Torresdey et al., 1996; Kapoor and Viraraghavan, 1997; Matheickal and Yu, 1997; Zhang et al., 1998; Sarret et al., 1999; McHale and McHale, 1994; Mashitah et al., 1999; Tereshina et al., 1999; Zhou, 1999). Fungi remove metals essentially by adsorption, chemisorptions (ion exchange), complexation, coordination, chelation, physical adsorption and microprecipitation (Guibal et al., 1995; Huang and Huang, 1996; Kapoor and Viraraghavan, 1997; Sarret et al., 1998). There are also possible oxydo-reduction taking place in the biosorbent. When metals are removed by ionic exchange, they generally replace K^+, Mg^{2+}, Ca^{2+} and H^+ contained in biomasses (Akthar et al., 1995; Zhou, 1999; Gomes et al., 1999; Mashitah et al., 1999). Table 1 gives a synthesis of some works on metals removal from wastewaters by some fungi. Biomasses used to remove metals from wastewaters are generally produced against few residual biomasses from fermentation (Fourest et al., 1994; Meyer and Wallis, 1997). Metals sequestrations by fungi are influenced by the mineral and organic compositions content of the medium in which biomasses are produced. Biomasses granulometries and physiological states (living or dead), co-ions, metals concentrations and physical parameters (temperature, pH, ionic force, presence of others metals) influence also metals removal from polluted waters (Volesky, 1994; Akthar et al., 1995; Gomes and Linardi, 1996; Modak et al., 1996; Gardea Torresdey et al., 1997; Yu and Kaewsan, 1999; Zhou, 1999). Metals by fungi from various raw effluents (gold mining effluent, tanning effluent, swine water, polluted lake waters) are sometimes completely removed (see Table 1). However, these outputs depend on the metal and fungus involved.

To increase fungal biomasses removal capacities, some of them undergo physicochemical treatments (soda or acidic treatments, insertion of functional groupings, heat treatment) (Akthar et al., 1995; Akthar and Mohan, 1995; Kapoor et al., 1999; Kramer and Meisch, 1999; Yin et al., 1999; Yan and Viraraghavan, 2000). Moreover, *A. niger* biomass treatment by soda, makes it possible to adsorb 2.5 to 1000 mg Ag l^{-1} of Ag^+ in polluted water (Akthar et al., 1995). In the same way, Kapoor et al. (1999) obtained with a soda treated biomass of *A. niger*, the removal rates of Cd^{2+}, Cu^{2+} and Pb^{2+} superiors to that of activated carbon (F-400). Akthar and Mohan (1995) used the same type of biomass like precedent authors, and they obtained the removal rates of Zn^{2+} and Cd^{2+} superiors to that of Dowex-50 resin. An insertion of carboxyl and amino groups in *A. niger* biomass walls, makes it possible to obtain an adsorption rate ranging in the order of 172-1064 mmol (kg biomass)$^{-1}$ for Cd^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} (Kramer and Meisch, 1999). A simple detergent and alkaline solutions treatment of *M. rouxii* biomass was sufficient to obtain an increase in its adsorption capacity (Yan and Viraraghavan, 2000). Fungal biomasses that have sequestered metals can be regenerated following their washing with HNO_3 (0.05 N) and/or with Ca^{2+}, Mg^{2+} and K^+ (0.1 M) (Akthar et al., 1995, 1996; Kapoor et al., 1999).

DISCUSSION

Essential works on fungal utilization for raw wastewaters biopurification have been laboratory tests. This situation can be explained by the fact that fungal utilization in environmental biotechnology is still under investigation to assess information's on process implementation. To gain confidence with the results, these investigations are performed on synthetics wastewaters. The good results obtained in laboratory tests depend on growth medium optimisation (addition of co-substrate, nutrients, mediators molecules, physical parameters optimisation) and a good handling of biomasses. However, these works amongst other things prove some advantages when a mycoreactor is introduced in effluents treatment lines. In fact, there are some reductions of bactericidal effects and an increase in biogas production. Consequently work on pilot and the development of treatment plants are to be encouraged.

The degradation and the mineralisation of some recalcitrant dyes and organochlorinated compounds are effective by certain white rot fungi. However fungal aptitudes for raw wastewaters remain dependent on salts concentration, culture conditions and especially on the amendment of carbon and nutriment sources. Among the co-susbstrates tested for effluent pretreatment by fungi, glucose and sucrose were the best, when they were used at 5 to 10 g l^{-1}. To minimise the mycoreactor integration cost in the treatment line, the co-substrate could be provided by feeding the reactors with amylaceous effluents or others wastewaters rich in sucrose or glucose as these carbon sources proved to be the best co-substrate. About the growth medium impact on fungal capacities to decolourise HTL, one could use *C. hirsutus* in post processing of an activated wastewater, because of its sensitivity to organic nitrogen. The oxidoreductases activities could be more significant, thanks to the use of substrate that could ensure the role of mediators' molecules and guarantee the generation of $H_2\grave{O}_2$ in the reaction medium. Salts constraint could be overcome while proceeding to the desalinisation of the effluents before their treatment with fungi.

As regards metal removal, a standardization of adsorption rates unit and the rigorous description of biomass morphology (pellet diameter) will allow a better comparison of fungal capacities and a guide for the best choice of fungi. Biomass grinding to produce small particles and their engineering to increase their capacities to remove specific metals are promising way for fungal biosorption. Utilization of residual biomasses from fermentation is still minimal nowadays (Knapp and Newby, 1999); one needs to encourage such practice, because this constitutes a way of making use of them.

CONCLUSION

This review highlighted the capacities of certain fungi to pretreat raw wastewaters. However, essential works on this subject are still laboratories tests and they are of less industrial scale application. The white rot fungi are suitable for the degradation of a large variety of pollutants and to produce at the same time metabolites of great added values (proteins, enzymes). However, an optimisation of the culture media in carbon sources or nutrients and mediators molecules is very important to obtain a good output of pollutants degradations. With regard to other fungi, those also contribute to effluents purification with proteins and enzymes productions (for example, *A. awamory* and *A. niger*). Some residual biomasses from fungal fermentation, have been used to remove metals and dyes from effluents. Ultimately, the fungal biomasses present many assets for biopurification of wastewaters.

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