Chapter 4
Application of Biosorption and Biodegradation Functions of Fungi in Wastewater and Sludge Treatment

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4.1 Introduction

Sewage treatment has always been one of the core problems of environmental protection as wastewaters contain a variety of harmful substances such as heavy metals, dyes and phenolics (Rangabhashiyam et al. 2014). Disposing the sludge generated from sewage treatment plant in a safe way is also challenging. The surface and ground waters in many parts of the world have been subject to pollution due to the emission of industrial wastewater and cannot be used as drinking water (Rangabhashiyam et al. 2014). For the basic needs of life, there is the increasing need of pollution control and water quality protection. Various water treatment methods using physical and chemical techniques have been applied. The main methods include: (i) filtration (Zouboulis et al. 2002), (ii) ion exchange (Kabsch-Korbutowicz and Krupinska 2008), (iii) solvent extraction (Lin and Juang 2002), (iv) advanced oxidation processes (Esplugas et al. 2002), (v) activated carbon adsorption (Kurniawan et al. 2006). But most of technologies mentioned above are limited due to their high cost. Biological treatment is a relatively economical when comparing to the traditional physical and chemical processes (Crini 2006). Biological technologies such as biological adsorption and microbial degradation are commonly applied to the treatment of industrial effluents because many microorganisms such as bacteria and fungi are able to concentrate selected substances and degrade different contaminants (Fu and Viraraghavan 2001a; McMullan et al. 2001; Volesky 2007; Chen et al. 2014a; Wang et al. 2015). Utilizing fungi for biological treatment have been extensively studied due to their vast amounts of biomass generated from fermentation industries (Zhou and Kiff 1991). On the fungal cell wall there exist many functional groups such as carboxyl,

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hydroxyl, amino, sulfonate, and phosphonate, which bring about the excellent adsorption property in fungi (Cabib et al. 1988; Bowman and Free 2006). Fungi also possess multiple mechanisms for degradation of organic and inorganic pollutants (Awasthi et al. 2014; Mishra and Malik 2014b). Thus, fungi are playing increasingly important roles in wastewater treatment. The following notable features of fungi made them excellent candidates for treatment processes: (i) high adsorption capacity, (ii) easy solid–liquid separation, (iii) good adverse resistance, and (iv) broad degradation ability. The main goal of this review is to provide up-to-date information pertaining to the application of biological adsorption and biodegradation functions of fungi in sewage treatment.

4.2 Biosorption Function

Many literatures have reported the fungal application for wastewater treatment in recent years. Filamentous fungi are promising materials to replace or supplement traditional treatment processes (Sharma et al. 2011). Many genera of fungi have been researched both in living or inactivated form (Srinivasan and Viraraghavan 2010). For living cells, the mechanism involves biosorption and biodegradation because fungi can produce laccase or other enzymes to mineralize organic pollutants (Raghukumar et al. 1996). For dead cells, the mechanism is biosorption without active metabolic transport process (Volesky 2007).

4.2.1 Performance of Wastewater Treatment for Dyes, Heavy Metals and Phenolic Compounds

The ingredient of wastewater is usually very complex, containing all kinds of pollutants. This paper mainly focuses on fungal biosorption effect on organic dyes, heavy metals and phenolic compounds.

(1) **Dyes**

Dye wastewater is one of the most difficult industrial wastewaters to treat. Particularly from the textile industry, more than 1.5×10^8 m^3 of colored effluents are discharged annually (Ip et al. 2010). These dyes may be mutagenic or carcinogenic in human beings and could lead to dysfunctions of the liver, kidneys, central nervous and reproductive system (Dincer et al. 2007; Shen et al. 2009). It is also recognized that color is the first obvious sign of sewage. The presence of trace amounts of dyes in water can be easily visible and undesirable (Banat et al. 1996; Robinson et al. 2001). Activated carbon is an effective adsorbent which was widely used for dye removal in sewage treatment with several advantages such as the large surface area and high adsorption capacity. But the usage of activated carbon would be limited in many cases because of its disposal problem and the high running cost (Xiong et al. 2010). The application of fungi for the removal of dyes is an attractive alternative to the colored sewage treatment (Solis et al. 2012). Fungal species such as *Pencillium oxalicum*, *Aspergillus niger*, *Trametes versicolor*, *Rhizopus stolonifer*, *Rhizopus oryzae*. have been widely reported as biosorbents for the removal of dye from aqueous solution (Abd El-Rahim et al. 2003; Bayramoğlu and Arica 2007; Binupriya et al. 2007; Srinivasan and Viraraghavan 2010; Solis et al. 2012; Akar et al. 2013; Rangabhashiyam et al. 2014). The representative examples of dye decolorization by fungi are tabulated in Table 4.1. Since color is the first obvious sign of the presence of sewage, the decolorization rate, i.e., the removal percent becomes one of the most important indices for colored wastewater treatment. From the table it is obvious that a wide variety of fungi are capable of decolorizing all kinds of dyes. With regard to a certain type of dye, different species of fungi tend to have different adsorption effectiveness. For example, the removal efficiency of Reactive Black 5 can exceed 99 % after 48 h treatment by using *Penicillium geastrivorus* (Yang et al. 2003), but the removal rate drops to 88 % when the adsorbent is change into *Aspergillus niger* over 60 h (Taskin and Erdal 2010), which suggests that dye adsorption in fungi is species specific. Meanwhile, some special types of fungi are found to have high biosorption effectiveness on multiple dyes that make them potential candidates to treat colored wastewater generated from industry contain a variety of dyes. For instance, mycelial pellets formed of marine-derived *Penicillium janthinellum* P1 have a broad spectrum of adsorption capacity (Fig. 4.1): its decolouration efficiency of Congo Red, Naphthol Green B, Eriochrome Black T, Amino Black 10B could exceed 99 %, and the removal rate of Neutral Magenta, Methyl Red, Acid Fuchsin, Crystal Violet, and Brilliant Green could reach up to 94.4, 82.1, 63.5, 56.9, and 63.0 %, respectively (Wang et al. 2015).

(2) **Heavy metals**

In addition to dyes, the existence of heavy metals in wastewater presents further complexity in processing. Industries that discharge heavy metals sewage includes: electroplating, battery manufacturing, mining engineering, printing, photography industry (Kadirvelu et al. 2001). Heavy metals in the environment may accumulate in the food chain and eventually cause great harm to human health (Kurniawan et al. 2006; Dal Bosco et al. 2006; Fu and Wang 2011). Although many biological materials can adsorb heavy metals, only those with sufficiently high metal-binding capacity and selectivity for heavy metals are appropriate for use in the biosorption process (Sag 2001). Fungal cell wall surface contains different functional groups, many of which are found to play vital roles in metal chelation (Rangabhashiyam et al. 2014). Fungi have a large capacity for heavy metal sorption from aqueous solutions and in certain circumstance even outperformed activated carbon (Rangabhashiyam et al. 2014). Fungal species such as *Aspergillus niger*, *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Rhizopus arrhizus*, *Trametes versicolor,* and *Fusarium* sp. have been extensively researched in the removal of heavy metal ions as they are abundantly available and low cost in mass production. Biosorption capacities of heavy metal ions on various fungal species are compared in Table 4.2.

Table 4.1 Results of dye decolorization by fungi

Fungi	Dye	Percent removal (%)	Initial dye concentration (mg/L)	Time of contact	References
Rhizopus oryzae	Rhodamine B	90	100	5 h	Das et al. (2006)
Pencillium oxalicum	Reactive Blue 19	91	100	80 min	Zhang et al. (2003)
Aspergillus niger 31	Polar Red	94	300	8 d	Abd El-Rahim et al. (2003)
	Direct Blue 1	63.2	800	6 h	Bayramoğlu and Arica (2007)
Aspergillus niger	Acid Blue 29	80	50	30 h	Fu and Viraraghavan (2001b)
Penicillium geastrivorus	Reactive Black 5	>99	100	48 h	Yang et al. (2003)
Aspergillus niger	Reactive Black-5	88	100	60 h	Taskin and Erdal (2010)
Penicillium chrysogenum MT-6	Reactive Black-5	89	300	100 h	Erdal and Taskin (2010)
Trametes pubescens	Congo Red	98	100	60 min	Si et al. (2015)
Phanerochaete chrysosporium	Amido black 10B	98	1000	3 day	Senthilkumar et al. (2014)
Aspergillus lentulus	Acid Blue 120	90	100	12 h	Kaushik et al. (2014)
Thamnidium elegans	Reactive Red 198	98	100	75 min	Akar et al. (2013)
Penicillium janthinellum P1	Congo Red	>99	150	24 h	Wang et al. (2015)
	Naphthol Green B	>99	150	24 h	
	Eriochrome Black T	>99	150	24 h	
	Amino Black 10B	>99	150	24 h	
	Neutral Magenta	94.4	150	24 h	
	Methyl Red	82.1	150	24 h	
	Acid Fuchsin	63.5	150	24 h	
	Crystal Violet	56.9	150	24 h	
	Brilliant Green	63.0	150	24 h	

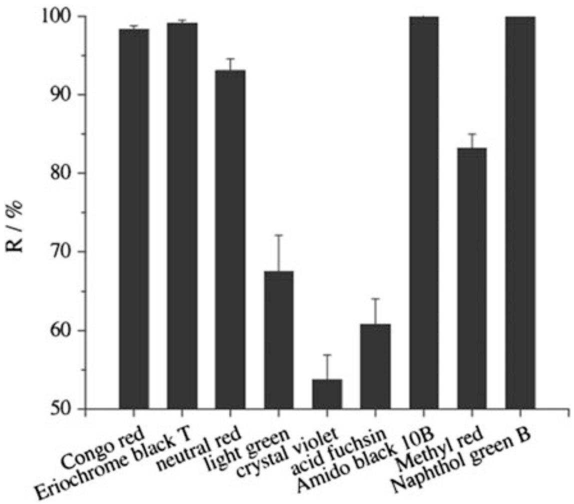

Fig. 4.1 The decolorization rate of *Penicillium janthinellum* P1 in treating nine different dyes (Wang et al. 2015)

The maximum hexavalent chromium adsorption capacities are ranging from 10.75 to 117.33 mg/g by different species of fungi, which shows the specific differences.

(3) **Phenolic compounds**

Phenol can cause harm to human health even in minute quantity (Senturk et al. 2009; Hank et al. 2014), thus US Environmental Protection Agency have taken stringent measures to lower phenol content in the wastewater to <1 mg/L (Banat et al. 2000). Many other phenolic compounds also have different degrees of toxicity, which are contained in sewages originated from petrochemical, phenol producing, coal conversion and other chemical processes (Hamdaoui and Naffrechoux 2007a, b). In recent years, many studies have focused on fungi that are able to biosorb phenols and chlorophenols. Table 4.3 shows the data on the biosorption capacities of phenol and phenolic compounds by various fungi.

4.2.2 Mechanisms

Due to the diversity of the fungi and the complexity of contaminants in wastewater,the mechanism of fungal biosorption is often difficult to characterize, except perhaps in the simplest laboratory systems where a variety of mechanisms may be operative under given conditions (Gadd 2009). There are variety of ways for the pollutant to be captured by fungal cell, thus biosorption mechanisms could be multiple and in many cases they are still not very well understood (Sag 2001). The biosorption mechanisms are classified to different types on the basis of cell metabolism status or pollutants sorption location (Fig. 4.2).

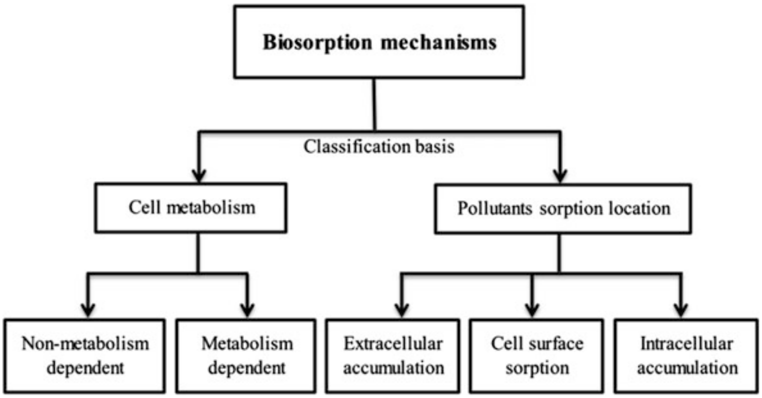

Fig. 4.2 The biosorption mechanisms are classified to different types on the basis of cell metabolism status or pollutants sorption location

Table 4.2 Biosorption capacities of heavy metals by fungi

Fungi	Heavy metal	Adsorption capacities (mg/g biomass)	References
Ganoderma lucidum	Zr(IV)	142.5	Hanif et al. (2015)
Coriolus versicolor	Zr(IV)	110.75	Amin et al. (2013)
Penicillium citrinum	U(VI)	127.3	Pang et al. (2011)
Rhizopus arrihizus	U(VI)	112.2	Wang et al. (2010)
Yarrowia lipolytica	Ni(II)	95.33	Shinde et al. (2012)
Pleurotus ostreatus	Cr(VI)	10.75	Javaid et al. (2011)
Aspergillus niger	Cr(VI)	117.33	Khambhaty et al. (2009)
Coriolus versicolor	Cr(VI)	62.89	Sanghi et al. (2009)
Rhizopus arrhizus	Cr(VI)	78	Aksu and Balibek (2007)
Saccharomyces cerevisiae	Cr(VI)	32.6	Ozer and Ozer (2003)
Surfactant-modified yeast	Cr(VI)	94.34	Bingol et al. (2004)
Trametes versicolor	Cu(II)	140.9	Subbaiah et al. (2011a)
Aspergillus niger	Cu(II)	20.91	Iskandar et al. (2011)
Penicillium simplicissimum	Cu(II)	16.18	Iskandar et al. (2011)
Trichoderma asperellum	Cu(II)	12.81	Iskandar et al. (2011)
Mucor rouxii	Zn(II)	53.85	Yan and Viraraghavan (2003)

(continued)

Table 4.2 (continued)

Fungi	Heavy metal	Adsorption capacities (mg/g biomass)	References
Trametes versicolor	Pb(II)	208.3	Subbaiah et al. (2011b)
	Cd(II)	166.6	
Amanita rubescens	Pb(II)	38.4	Sari and Tuzen (2009)
	Cd(II)	27.3	
Clitopilus scyphoides	Cd(II)	200	Moussous et al. (2012)
Auricularia polytricha	Cd(II)	63.3	Huang et al. (2012)
	Cu(II)	73.7	
	Pb(II)	221	

According to the correlation with the cell metabolism, fungal biosorption mechanisms can be divided into two types: (i) Non-metabolism dependent (passive uptake)—involving ion exchange, precipitation, complexation and physical adsorption (Veglio and Beolchini 1997), and (ii) Metabolism dependent (active uptake)—comprising an energy-driven process (Gadd 2009). Biosorption by dead fungal cells is a passive process which based on the interaction between the cell biomass and adsorbate. Dead cells capture pollutants through chemical functional groups on the cell wall which takes up most of the cellular dry weight. Passive uptake could also be present when the cell is metabolically active, however, it may be suppressed by cellular protective mechanisms against the toxic pollutants, e.g., active metal exclusion processes (Volesky 2007). Thus the dead fungal biomass holds promising biosorption capacity towards the toxic pollutants such as heavy metals and phenolic compounds (Kumar et al. 2008; Rao and Viraraghavan 2002). When living cells are used, the biosorption mechanisms become much more complicated. A lot of mechanisms may exist simultaneously since many cell metabolisms may be involved in biosorption. In addition, a variety of reaction, such as (i) adsorption, (ii) ion exchange, (iii) complexation, and (iv) precipitation may be affected by the change of the microenvironment around the cells, which can be altered by fungal cellular metabolism; for instance: nutrient uptake, respiration and metabolite release (Gadd and White 1993).

According to the location where the pollutants were captured and concentrated, fungal biosorption may also be classified as: (i) extracellular accumulation or precipitation, (ii) cell surface sorption (e.g., ion exchange, complexation, physical adsorption, precipitation), and (iii) intracellular accumulation (e.g., transport across cell membrane) (Muraleedharan et al. 1991). The chemical constitution and structural organization of the fungal cell wall are very complicated and all kinds of pollutant can either be bound in its surface or be deposited within its structure before they entry into the cytoplasm where they could be detained by other compounds or organelles. Fungal cell wall consists mainly of polysaccharides, proteins and lipids, offering diverse function groups, such as carboxyl (–COOH), phosphate (PO_4^{3-}), hydroxyl (–OH), amino (–NH_2), thiol (–SH) (Crini 2006) that are able to interact with adsorbed contaminants in different degrees. Many species of fungi have microfibrillar layer structures inside the

Table 4.3 Biosorption capacities of phenolic compounds by fungi

Fungi	Phenolic compounds	Adsorption capacities (mg/g biomass)	References
Aspergillus niger	Phenol	0.5	Rao and Viraraghavan (2002)
Emericella nidulans	2,4-dichlorophenol	9.1	Benoit et al. (1998)
	p-chlorophenol	3.0	
Rhizopus arrhizus	Pentachlorophenol	14.9	Bell and Tsezos (1987)
Trametes versicolor	Phenol	50	Kumar et al. (2009)
	o-chlorophenol	86	
	p-chlorophenol	112	
Schizophyllum commune	Phenol	120	Kumar and Min (2011)
	o-chlorophenol	178	
	p-chlorophenol	244	
Pleurotus sajor-caju	Phenol	89	Denizli et al. (2005)
	o-chlorophenol	159	
	p-chlorophenol	188	
	2,4,6-trichlorophenol	372	
Phanerochaete chrysosporium	Phenol	115	Denizli et al. (2004)
	o-chlorophenol	190	
	p-chlorophenol	228	
	2,4,6-trichlorophenol	421	

cell wall, which are composed of chitin or cellulose chains. The chitosan plays very important roles in fungal biosorption. Heavy metal ions could bind to the amine sites of chitin (R_2–NH) and chitosan (R–NH_2). Meanwhile, these amine sites also appear to be the major reactive groups for dyes, since intermolecular interactions of the dye molecules are most probable in chitosan–dye systems (Crini and Badot 2008). The Mucorales family (e.g., *Rhizopus arrhizus*), have outstanding biosorbent performances, which may be attributed to the high chitin content in their cell walls.

The diversity of chemical structures encountered in organic pollutants meant that their molecular size, charge, solubility, hydrophobicity, and reactivity, all affect the wastewater composition, choice of biosorbent and their biosorption efficiency. Pragmatically, of course, it may not be necessary to understand what mechanism is operative if the prime research goal is to identify an efficient biosorbent system (Gadd 2009).

4.2.3 Factors Influencing Biosorption Capacities

A variety of factors can affect biosorption. The type and nature of the fungal biomass or derived product can be very important. The properties of the biomass can be influenced by the age and growth condition of the fungal cells, which lead to changes in cell size, cell wall components, extracellular secretion, and other metabolic activities. Changes of cellular properties can also be achieved by physical and chemical pretreatment. The environment of biosorption and the use of bioreactors would dramatically influence the reactions between the biomass and contaminants thereby alter the biosorption efficiency.

(1) **Pretreatment for fungal biosorbents**: **physical or chemical treatment**

Physical processing of fungal biomass usually includes autoclaving, drying and crushing. Many researches in the biosorption of organic compounds, heavy metals or other toxicity pollutants with fungal biomass showed that enhanced sorption capacity was obtained using dead fungal biomass rather than the living cells (Rao and Viraraghavan 2002; Kumar et al. 2008). That may be because the living fungi can prevent toxic substances from entering into cells by cellular protective mechanisms. Physical treatments such as boiling, drying, lyophilisation or autoclaving will kill the fungal cells and improve the efficiency of sorption accordingly. Autoclaving could also rupture the fungal structure and expose the potential binding sites for certain adsorbate (Fu and Viraraghavan 2000). Drying can bring convenience to storage and transportation of fungal biomass and crushing will enhance the surface area so as to improve the adsorption rate.

Chemical treatments such as alkali treatment can improve biosorption capacity in some circumstances: chitin deacetylation resulting in the formation of chitosan-glucan complexes with higher metal affinities compare to the control group (Wang and Chen 2006). Acid pretreatment could change the negatively charged surface of fungal biomass to positively charged and thus increasing the attraction between fungal biomass and anionic dyes (Fu and Viraraghavan 2001b). In practical application, the specific pretreatment methods are determined by the types of adsorbates and fungal species. For instance, acid and alkali treatment decrease the adsorption capacity of Congo red onto fungal biomass of *Trametes versicolor*, while autoclaving could improve the removal percentage (Fig. 4.3).

(2) **The environment of biosorption**: **pH, temperature and salinity**

The solution pH value determines the surface electrical charge of fungal biomass and the ionic forms of contaminants. Therefore, solution pH affects both adsorbate chemistry and the fungal biomass binding sites. Heavy metal biosorption is strongly pH-dependent in almost all systems examined. Competition between cations and protons for binding sites leads to inferior biosorption efficiency of metals like Cu, Cd, Ni, Co, and Zn at low pH values (Gadd and White 1985; Shroff and Vaidya 2011). Conversely, anionic metal species like TcO_4^{-}, $PtCl_4^{3-}$, CrO_4^{2-}, SeO_4^{2-}, $Au(CN)_2^{-}$ may have a higher absorption rate at lower pH values. There also exists competition between cations, which can depress the biosorption of the metal. For dye biosorption, the decolorization rate usually has higher value when pH is lower (Fig. 4.3). For instance, the biosorption of Reactive Red 120 dye on the fungal biomass *Lentinus sajor-caju* increased as the pH was decreased, and similarly, maximum removal of reactive dye Remazol Black-B was found in the range of pH 1–2 and dropped sharply at higher values (O’Mahony et al. 2002; Arica and Bayramoğlu 2007).

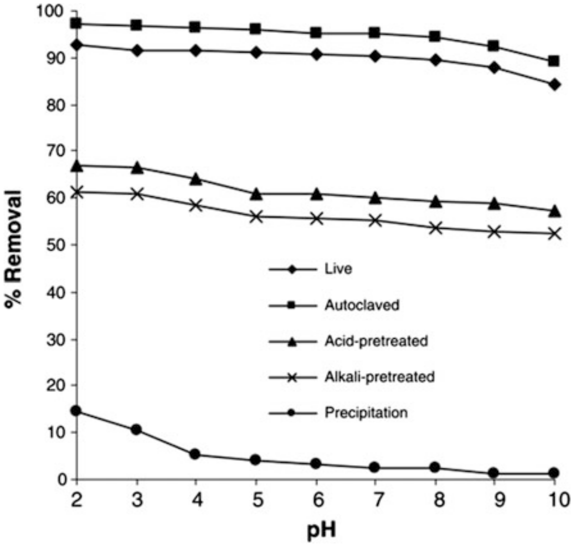

Fig. 4.3 Effect of pH on biosorption of Congo red fungal biomass of *Trametes versicolor* pretreated by different methods (Binupriya et al. 2008)

The effect on biosorption of temperature does not appear to be as strong as pH values. In some cases, higher temperature would enhance the biosorption efficiency due to the increase of the surface activity of fungal biomass and the kinetic energy of dye (Bakshi et al. 2006; Kaushik and Malik 2009). But at relatively high temperature, e.g., above 40 °C, the biosorption capacity often decrease in many cases (Iqbal and Saeed 2007; Erden et al. 2011), possibly due to the deactivation of the cell surface and destruction of some binding sites. Low temperature could restrain living cell metabolism systems and most of auxiliary processes which aid biosorption resulting in the decrease of biosorption efficiency (Gadd 2009).

Certain types of industrial wastewaters contain high salt concentration which may influence the biosorption processes. For example, the addition of 50 g/L salt resulting in a 28.8 % reduction in the biosorption capacity of Yellow RL dye of the *Rhizopus arrhizus* biomass (Aksu and Balibek 2010). However, some marine-derived fungi, like *Penicillium janthinellum* ZJU-BS-P1, have a strong tolerance with high salt concentrations (Fig. 4.4).

In addition to the above factors, the ionic strength, initial pollutant concentration and sorbent dosage would also affect the biosorption efficiency to a certain extent (Zhou and Banks 1993; Asgher et al. 2008; Khelifi et al. 2009; Levin et al. 2010).

(3) Biomass immobilization

The immobilization of fungal biomass may enhance biosorption capacity due to: (i) improved mechanical strength, (ii) increased porosity characteristics, (iii) less clogging, (iv) ease for regeneration and (v) multiple biosorbent recycle (Aksu and Gonen 2004; Aksu 2005; Solis et al. 2012). A number of materials have been successfully applied to immobilize fungal biomass. For example, nylon sponges, polyurethane foam, Luffa sponges, polystyrene foam, Ca-alginate beads, lignite

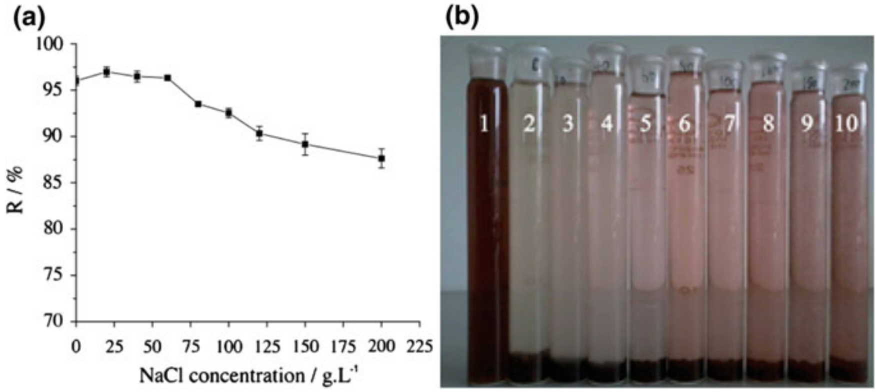

Fig. 4.4 **a** Effects of NaCl concentration on the biosorption of Congo red by marine-derived *Penicillium janthinellum*. **b** Congo red solutions after 24 h treatment (lane 1: control solution, lane 2–10: NaCl concentration 0, 20, 40, 60, 80, 100, 120, 150 and 200 g L^{-1}) (Wang et al. 2015)

granules, $ZrOCl_2$-activated pumice (Karimi et al. 2006; Maurya et al. 2006; Iqbal and Saeed 2007; Bohmer et al. 2010; Enayatzamir et al. 2010; Grinhut et al. 2011). Kocaoba and Arisoy (2011) observed that the biosorption capacity of *Pleurotus ostreatus* immobilized on Amberlite XAD-4 to remove Cr(III), Cd(II) and Cu(II) remained stable even after 10 cycles of sorption and desorption.

4.3 Biodegradation Function

Biodegradation is a kind of wastewater treatment that tackles the pollutants more thoroughly than biosorption and has a wide range of applications. In contrast to biosorption that can use dead fungal cells (and sometimes their effect is even better than living cells), biodegradation must be performed by living cells since the degradation process is controlled by enzymes secreted by fungi. Meanwhile, the wastewater sludge processing is one of the important applications of fungal degradation.

4.3.1 Biodegradable Application in Sludge Treatment

(1) **Roles of fungal laccases**[1]

Among the different types of enzymes produced by fungi, laccase is one of the best researched. It is a multicopper oxidase glycoprotein that is well known to be

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[1] Additional information on the role of fungal laccase in wastewater treatment can be found in Chap. 5—*Potential of white-rot fungi to treat xenobiotic-containing waster* and Chap. 6—*Fungal bioremediation of emerging micropollutants in municipal wastewaters*.

ubiquitous in all kinds of living organisms. Fungal laccases play important roles in catalyzing the oxidation of a wide range of environmental pollutants, such as lignin, dyes and phenolic compounds (Aust and Benson 1993; Xu 1996; Giardina et al. 2010). Laccases consist of a sequence of polypeptide of about 500 amino acid residues and are linked to saccharides. Most fungal laccases are extracellular secretions and their molecular weights range is about 60–70 kDa (Baldrian 2006; Giardina et al. 2010). Laccases are nonspecific enzymes to their substrates, thus they are able to catalyze the oxidation of many organic contaminants including phenols (Reiss et al. 2011). They have also been used for the decolorization and detoxification of effluents from textile and paper making industries (Harms et al. 2011). As shown in Table 4.4, laccases has been used for the removal of many emerging organic contaminants from wastewater treatment plant effluents (Gasser et al. 2014a). For instance, laccases from *T. versicolor* could remove natural and synthetic estrogens, including estrone and 17β-estradiol, estriol from municipal wastewater (Auriol et al. 2007, 2008). In another batch study, *T. versicolor* was found to be able to remove 95–100 % of oxybenzone (Garcia et al. 2011). *Funalia trogii* ATCC 200800 had a strong ability to mineralize synthetic dyes by producing the laccase or MnP (Raghukumar et al. 1996). Laccase from *Trametes* sp. had been applied to biodegrade phenolic endocrine-disrupting chemicals including bisphenol A, nonylphenol, octylphenol, and ethynylestradiol (Tanaka et al. 2001).

(2) Sludge treatment

Traditional wastewater treatment generates plenty of sewage sludge which must be disposed of to maintain environmental protection (Zaidi 2008). Sludges contain

Table 4.4 Pollutants removal by laccases in various effluents

Sources of fungal laccase	Pollutants investigated	Percent removal (%)	References
Trametes versicolor	E1, E2, E3, EE2	100	Auriol et al. (2007)
Trametes versicolor	E1, E2, E3, EE2	97	Auriol et al. (2008)
Thielavia genus	BPA	98	Hommes et al. (2012)
Coriolopsis polyzona	BPA	93	Hommes et al. (2012)
Myceliophthora thermophila	E1	98	Lloret et al. (2013b)
	E2	≥97	
	EE2	≥99	
Coriolopsis polyzona	BPA	90	Demarche et al. (2012)
Coriolopsis gallica	BPA	≥85	Nair et al. (2013)
	diclofenac	30	
	EE2	≥85	
Thielavia genu	BPA	~66	Gasser et al. (2014b)

E1 estrone; *E2* 17 β–estradiol; *E3* estriol; *EE2* 17 α–ethinylestradiol; *BPA* bisphenol A more than 90 % of water along with organic solids that are problematic during transportation and treatment process (More et al. 2010). Therefore, the recovery and disposal of sludge is also an important issue in sewage treatment (Martins et al. 2004). The wastewater sludge contains a variety of microorganisms and organic matter. Fungi are saprophytic organisms and their nutrient requirement can be accomplished by the degradation of sludge. (Osiewacz 2002; Fakhru'l-Razi and Molla 2007). Filamentous fungi have great potential for sludge treatment and their functions include: (i) organic solids reduction, (ii) bioflocculation, (iii) pathogens removal, (iv) dewaterability, and (v) detoxification; a detailed account was given by More et al. (2010). Fungi have some advantages over bacteria in sludge treatment because of their strong capability to degrade more complex and variety of substrates (Khursheed and Kazmi 2011). Various fungi have been used for sludge treatment. For example: *Aspergillus niger* (Mannan et al. 2005), *Phanerochaete chrysosporium* (Molla et al. 2001), *Penicillium expansum* (Subramanian et al. 2008), *Trichoderma* sp. (Verma et al. 2005).

(3) **Extensive applications**

Residues of pesticides in the wastewater are harmful to the environment. For example, triclosan, which is a powerful bacteriostat, has been used extensively in soaps, shampoos, toothpastes, and disinfectants. It has long half-life and may potentially cause long term health risks in human body. Triclosan biodegradation yield can reach 71.91 % at about 7.5 mg/L initial concentration in semi-synthetic medium by using *Aspergillus versicolor* (Tastan and Donmez 2015). Coking wastewater contains various phenolic compounds and many other contaminants that are refractory, toxic and carcinogenic. When treated with *Phanerochaete chrysosporium*, a white-rot fungus, the removal rates of phenolic compounds and COD (Chemical Oxygen Demand) can achieve 84 and 80 %, respectively in 3 days (Lu et al. 2009). It is also found that laccase from a *Trametes* species has ability of degradation on polyunsaturated fatty acids and conjugated resin acids (Zhang et al. 2005). Thus, selected fungi and enzymes are also used for pitch removal (Singh and Singh 2014).

4.3.2 Factors Influencing Fungal Biodegradation

Various physicochemical operational parameters, including temperature, pH, nutrition, redox mediator, and the type of bioreactor, can influence the efficiency of fungal biodegradation.

(1) **Temperature, pH and nutrition**

Temperature is an important factor for all processes associated with fungal vitality. The maximum rate of biodegradation is generally related to the optimum growth temperature for each fungal species. It is worth mentioning that the oxidations catalyzed by laccases usually occur at ambient temperature (20–40 °C), thus laccases have become green and environmentally friendly for the elimination of pollutants (Wells et al. 2006). The pH has a great effect on the efficiency of biodegradation by influencing the enzyme activity. Fungi and yeast often show better biodegradation activities at acidic or neutral pH than bacteria (Khan et al. 2013). The effects of pH are also related to the transport of pollutant molecule across the cell membrane, which is considered as the rate limiting step for the biological catalysis (Kodam et al. 2005). For example, the removal rates of phenolic compounds and COD from coking wastewater by immobilized fungus *Phanerochaete chrysosporium* was significantly affected by pH and temperature (Fig. 4.5).

Enzymes production is dependent on the supply of nutrients to a certain degree. Certain types of wastewater, such as printing and dyeing effluents, lack nutrition, and require additional nutrients to improve the degradation efficiency of the fungi. Many studies on the azo dye decolorization were performed in the presence of additional carbon and nitrogen sources. The addition of glucose has frequently been demonstrated to improve the efficacy of azo dye degradation (Khan et al. 2013). But carbon sources seemed to be less effective than nitrogen sources (such as peptone, urea and yeast extract) in promoting biodegradation, probably due to the fact that nitrogen is the necessary building block of protein synthesis. Nitrogen sources can also regenerate NADH, which acts as an electron donor for the reduction of contaminants by microorganisms (Chang et al. 2000).

(2) **Mediator**

Laccases and other oxidases are able to oxidize small chemical compounds leading to radical formation (Canas and Camarero 2010). These radicals can act as redox mediators oxidizing compounds that might otherwise not be oxidized, thus broadening the substrate range. Redox mediators can also enhance many reductive processes under anaerobic conditions (Kodam et al. 2005). For instance, complete diclofenac removal could be achieved at pH 4 after 4, 2, and 0.5 h using no mediator, syringaldehyde, and 1-hydroxybenzotriazole, respectively (Lloret et al. 2013a).

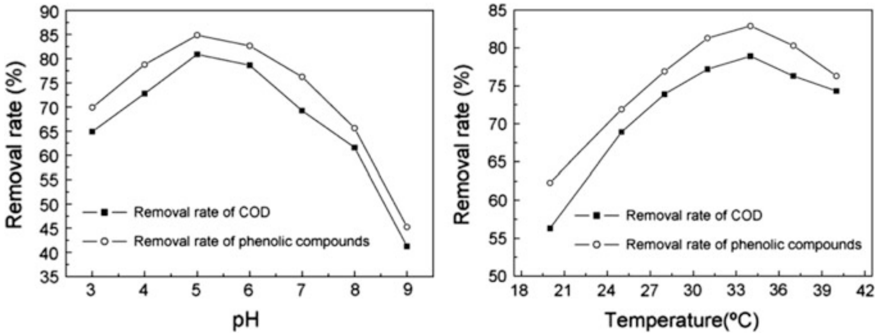

Fig. 4.5 Effect of pH on phenolic compounds removal rate (temperature 30 °C) and effect of temperature on phenolic compounds removal rate (pH 6.0) from coking wastewater by immobilized fungus *Phanerochaete chrysosporium*. Initial concentration of phenolic compounds and COD 313.5 and 3420 mg/L, respectively (Lu et al. 2009)

(3) **Use of bioreactors**

Bioreactor is an important tool for biodegradation. It can control the interaction pattern between fungal cells and pollutants in wastewater, thus influence the biodegradation efficiency greatly. Rotating drum, packed bed, fluidized bed, immobilized, and membrane bioreactors have been used as bioreactors. For instance, a membrane bioreactor using *Trametes versicolor* combined with reverse osmosis was effective for decolorization of dye wastewater (Kim et al. 2004). A wood-rotting fungal strain F29 decolorized 95–99 % Orange II in a continuous packed bed and fluidized bed bioreactor systems (Zhang et al. 1999). Immobilized bioreactors have been found to exhibit good biological activities and abilities for longtime operation (Srinivasan and Viraraghavan 2010).

4.4 Mix Fungi and Cooperation

Most studies demonstrate the effectiveness of a certain fungal strain to remove a particular contaminant; however, such specificity may limit the range of pollutants that can be treated by the fungus. Moreover, there are wide variations in the pollutant uptake capacity among different fungal strains. As industrial effluents contain various organic and inorganic contaminants, to develop a biological system capable of remediating all kinds of wastewaters, diverse types of microbial strains should be used in the form of a consortium (Mishra and Malik 2014a).

4.4.1 Cooperation Between Fungi

Different fungal strains usually possess different capacity to adsorb a specific pollutant. Therefore, the combination of fungi that exhibit different biosorption functions would enhance the total contaminants removal rate. For instance, a tripartite fungal consortium was studied for the abilities to remove metals (Cr^{6+} and Cu^{2+}) and dyes (Acid Blue 161 and Pigment Orange 34) from mixed waste streams (Mishra and Malik 2014b). The consortium consisted of *Aspergillus lentulus, Aspergillus terreus* and *Rhizopus oryzae* was significantly more effective than individual in removing the metals and dyes (Fig. 4.6).

The degradation function of fungi is very powerful, but there are large differences between the species. Therefore, the utilization of mix fungi has huge potential to improve the degradation efficiency. For instance, a mixed filamentous fungi culture (*Aspergillus niger* and *Penicillium corylophilum*) was used in a sewage sludge bioremediation study, and the highest removal of turbidity, total suspended solid and COD were achieved at 99, 98, and 93 %, respectively, by day 10 compared to the control (Rahman et al. 2014).

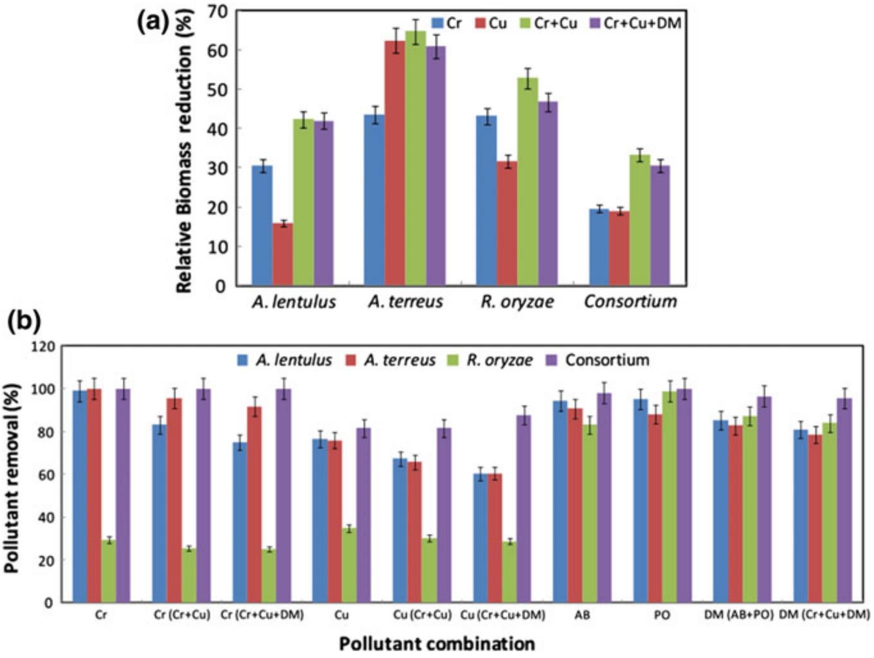

Fig. 4.6 **a** Performance of fungal consortium versus individual strain in terms of **a** relative biomass reduction and **b** pollutant removal from mixed pollutant. *AB* Acid Blue 161, *PO* Pigment Orange 34, *DM* dye mixture (Mishra and Malik 2014b)

During the liquid culture process, filamentous fungal could form mycelial pellets. As self-immobilized and bioactive particles, these pellets show advantages over mycelium for some industrial applications, such as: (i) strong surviving ability, (ii) fast settlement rates, (iii) easy solid–liquid separation and (iv) good reusability. Mycelia pellet can be used as a biological carrier for whole-cell immobilization due to its stable structural characteristics. As shown in Fig. 4.7, an innovative two-species whole-cell immobilization system was achieved simply by inoculating the *Pestalotiopsis* sp. J63 conidia into culture medium containing *Penicillium janthinellum* P1 pre-grown mycelia pellets and the resulting co-immobilization system was used for the treatment of paper mill effluent. Numerous insoluble fine fibers in the sewage were successfully and rapidly biodegraded and removed using this novel co-immobilization system (Chen et al. 2014a, b).

4.4.2 Cooperation Between Fungus and Bacterium

Bacteria have strong degradation ability on certain contaminants and they can be fixed by fungal mycelial pellets as biological carrier so as to form an immobilization

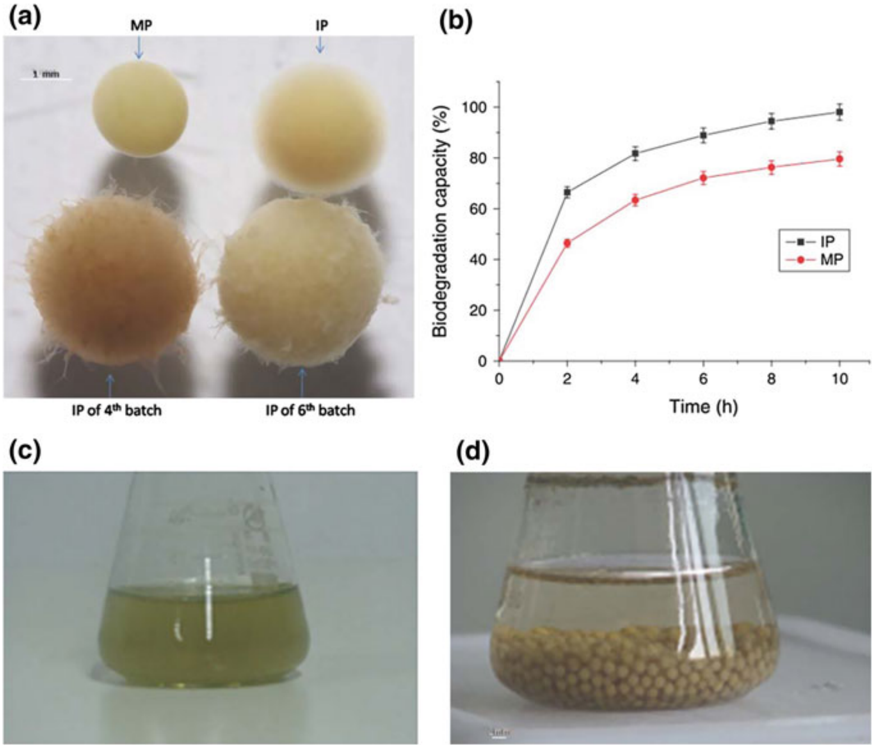

Fig. 4.7 The treatment of paper mill effluent by using a novel two-species whole-cell immobilization system. The immobilized pellets (IP) was made by inoculating the marine-derived fungus *Pestalotiopsis* sp. J63 spores into culture medium containing another fungus *Penicillium janthinellum* P1 pre-grown mycelia pellets (MP) for 2 days. **a** The different pellet sizes. **b** Biodegradation capacity of immobilized pellets and mycelia pellets in the process of wastewater treatment. **c** Diluted wastewater. **d** The effect of wastewater treatment using immobilized pellets. Modified and cited from Chen et al. (2014a)

system of fungi—bacteria that possesses multiple functions including biosorption and biodegradation. For instance, mycelial pellet of *Aspergillus niger* Y3 was used to immobilize the aniline degradation bacterium, *Acinetobacter calcoaceticus* JH-9 and other COD rapid removal bacteria. The combined mycelial pellets were applied in the SBR and the biological removal efficiency was about 0.9 mg aniline (L/d) (Zhang et al. 2011). A new azo dyes-decolorizing fungal strain *Penicillium* sp. QQ was used to immobilize *Sphingomonas xenophaga* QYY which has good azoreductase activity, the co-cultures were found to perform better than individual strains (Gou et al. 2009). Biosorption and direct biodegradation of polycyclic aromatic hydrocarbons (PAHs) in soil can be stimulated by *P. chrysosporium* and promoted synergistically by wild microorganisms (Chen and Ding 2012); the schematic diagram was shown in Fig. 4.8.

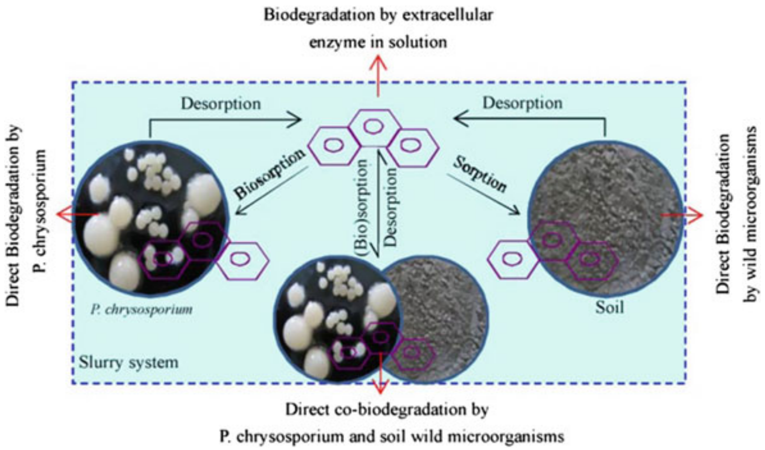

Fig. 4.8 The schematic diagram of (bio)sorption and biodegradation processes of PAHs in soil slurry systems containing *Phanerochaete chrysosporium* (Chen and Ding 2012)

4.5 Conclusions

Fungal biosorption and biodegradation of wastewater have received much attention as they are cost-effective methods for pollutants removal. The selection of the best treatment option for the harmless disposal of a certain type of industrial wastewaters is a difficult task because of their complex composition. The best way is often a combination of two or more species, and the choice of such consortium depends on the effluent composition, cost, toxicity of the degradation products and future use of the treated water (Solis et al. 2012). Most of researches on fungi in treatment of sewage and wastewater have been performed on a laboratory scale. Therefore, extensive laboratory works followed by series of pilot scale studies are essential for future industrial process applications.

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