Chapter 5
Biodegradation of Environmental Pollutants by Marine Yeasts

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Abstract Organic contaminants are among the main pollutants of ecosystems because of their presence in domestic, agricultural, or industrial effluents. Indeed, many organic xenobiotics such as aromatic hydrocarbons, pesticides, synthetic dyes, etc., are not easily biodegradable in the environment and can therefore accumulate in ecosystems causing various toxic symptoms in exposed organisms, including humans. Yeast-assisted biological treatment has emerged as a promising new strategy for the biodegradation of such hazardous contaminants. Firstly, this chapter provides an overview of the applications of yeast in the biodegradation of organic contaminants. Subsequently, synthetic dyes were chosen as a model of organic pollutants to highlight the enzymes involved in their biodegradation process using various yeast strains. Indeed, the main oxidases involved are laccase, tyrosinase, lignin peroxidase, and manganese peroxidase. While the main reductases are Azoreductase, NADH-DCIP reductase, and malachite green reductase. The last section highlights the effects of physicochemical conditions on the effectiveness of mycoremediation.

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5.1 Introduction

Yeasts are a group of polyphyletic fungi composed of basidiomycetes and ascomycetes species that have the particularity of living in a single-celled state. The environmental role that yeasts play is identical to that of other fungi. In fact, they act as saprophytes that transform plant and animal organic matter into biomass and its by-products (Kutty and Philip 2008). These ecological properties have been exploited by man since antiquity and continue to be developed even in the present day in a range of applications such as the fermentation processes (Beer, Wine, Sake, Soy Sauce), in food and feed ingredients (enzymes, flavors, pigments, amino acids, organic acids), the biocatalysis (pharmaceuticals, chiral chemical intermediates, and biotransformation), the biocontrol (food and feed safety, crop protection, and probiotics), as well as in fundamental research in biology and biomedical (Molecular biology, pathway engineering, systems biology mechanisms, drug metabolism and resistance, etc.) (Johnson and Echavarri-erasun 2011).

In addition to these applications, the exploitation of this ecological principle of yeasts in the biodegradation of different organic materials has prompted scientists to evaluate their capacity in the biodegradation of different carbon-based xenobiotics such as organic solvents, humic substances, phenolic compounds, petroleum, surfactants, pesticides, pharmaceuticals, and dyes, etc. (Aksu 2005). Numerous studies have reported the ability of yeast to biodegrade a variety of hazardous contaminants, including aromatic hydrocarbons (Deeba et al. 2018), phenol compounds (Filipowicz et al. 2020), pesticides (Han et al. 2019; Isia et al. 2019), fungicides (Kucharska et al. 2020), insecticide (Chen et al. 2012) and herbicides (States and States 2011) and synthetic dyes (Danouche et al. 2021c, 2022).

In this chapter, we first reviewed existing studies dealing with the topic of biodegradation of organic pollutants using halotolerant yeast strains. Then, we approached a comprehensive analysis of the enzymatic process involved by various yeast strains in the biodegradation of synthetic dyes as a model of organic pollution. Finally, we discussed the involvement of physicochemical factors in enhancing the mycoremediation capacity of yeast species.

5.2 Biodegradation of Organic Pollutants by Yeast

Biodegradation process is defined as an energy-dependent mechanism by which organic substances are decomposed into simpler and smaller by-products through the action of various enzymes (Kaushik and Malik 2009). This bioprocess is called mineralization when the products of the biodegradation are more straightforward elements, such as H_2O, CO_2, NH_3, CH_4, H_2S, or PO_3. This same process is defined

as biotransformation when the organic compounds are not completely mineralized (Danouche et al. 2021b). Numerous halophilic microorganisms belonging to bacteria, fungi, and microalgae have shown the ability to decompose a wide variety of organic hazardous substances under high salt conditions (Castillo-carvajal et al. 2014). In recent years, the biotechnological application of fungi (mycoremediation) has become a model example for bioremoval of organic contaminants. It has been reported that various species of fungal could be used for the biodegradation of organic chemicals including aromatic and aliphatic hydrocarbons, industrial dyes, and other organic contaminants, released into the aquatic environment from various industrial and agricultural sectors (Aleu and Collado 2009; Sen et al. 2016). Despite the advantages of yeast strains over other species of the fungal kingdom, including rapid growth, high plasticity, and the ability to adapt to adverse growth conditions (Jafari et al. 2014; Sen et al. 2016), only a few studies have been conducted on the use of halotolerant yeast strains for the biodegradation of organic pollutants.

Aliphatic and Aromatic Hydrocarbons The yeast species described as hydrocarbon degraders are mostly from the genera of *Yarrowia, Candida, Pichia, Debaryomyces, Sporidiobolus, Metschnikowia, Lodderomyces, Rhodosporidium, Leucosporidium, Rhodotorula, Stephanoascus, Sporobolomyces, Trichosporon*, and *Cryptococcus* (Csutak et al. 2010; Kumari and Abraham 2011; Jain and Bajpai 2012; Gargouri et al. 2015; Deeba et al. 2018). Recently, Hashem et al. (2018) reported that other yeast strains of *Meyerozyma guilliermondii KKUY-0214, Yamadazyma mexicana KKUY-0160, R. taiwanensis KKUY-0162, P. kluyveri KKUY- 0163, R. ingeniosa KKUY-0170,* and *C. pseudointermedia KKUY-0192* were approved for their ability to degrade both aromatic and aliphatic hydrocarbon.

Phenolic Compounds Pollution with phenolic compounds can occur in the soil as well as in water bodies, due to their presence in discharges from industrial, agricultural, or domestic activities (Anku et al. 2017). Species of the genus *Candida* are documented as yeast strains with the highest capacity to decompose a diverse range of phenolic compounds. For example, a yeast strain of *C. rugopelliculosa* was reported to be able to decompose various phenolic compounds such as phenol, bisphenol A, nonylphenol, 4-methylphenol, 4-ethylphenol, 4-tert-butylphenol, 4-tert-OP, 4-tert-, and isooctane (Huang et al. 2017). The phenol was also reported to be degraded by other strains of *C. tropicalis* (Gong et al. 2021), *C. tropicalis* PHB5 (Basak et al. 2019), *C. subhashii* A011, *C. oregonensis* B021, *Schizoblastosporion starkeyi-henricii* L012 (Filipowicz et al. 2020), and *R. kratochvilovae* HIMPA1 (Patel et al. 2017).

Pesticides, Fungicides, Insecticide and Herbicides Various agricultural practices lead to the release of organic contaminants into the soil and surface or ground water. Selected yeast strains can be used as biodegradation agents for these xenobiotics. The pesticides like diazinon or pendimethalin can be biodegraded with *Saccharomyces cerevisiae* (Ehrampoush et al. 2017) or *Clavispora lusitaniae* (Han et al. 2019) respectively. The biodegradation of fungicides such as propiconazole was illustrated through the use of yeast strains of *Aureobasidium pullulans, Rhodotorula glutinis, and Cryptococcus* sp. (Kucharska et al. 2020). Regarding the insecticide,

Chen et al. (2012) showed that *C. pelliculosa* was efficient in the biodegradation of Bifenthrin. Also, *C. xestobii* was documented to have a high biodegradation capacity of Metolachlor and Alachlor herbicides (States and States 2011).

Synthetic Dyes Biodegradation of synthetic dyes by yeast has also been documented in the literature using different yeast strains (Danouche et al. 2021b). The most commonly used yeast for the degradation of synthetic dyes are strains belonging to the phylum Ascomycetes, such as *Saccharomyces*, *Candida,* and *Pichia* species. While only a few studies have involved basidiomycetous yeast strains, namely *Trichosporon* and *Pseudozyma* (Pajot et al. 2014). In the following section, we will focus on synthetic dyes as a model of organic pollutants because of their different chemical characteristics that make them resistant to biodegradation in natural ecosystems, as well as because of their toxicity toward exposed organisms, including humans (Danouche et al. 2021a). In the remainder of this chapter, an in-depth review of the enzymatic mechanisms involved in the biodegradation of these chemicals by yeast is presented in detail.

5.3 Yeast Enzymes Implications in the Biodegradation of Synthetic Dyes

Synthetic dyes can be degraded enzymatically by yeast cells in either the extracellular or in the intracellular compartment. The most studied enzymes for the biodegradation of dyes by yeast are the oxidases, which are the class of enzymes that use oxygen (O_2) as an electron acceptor to catalyze the redox reaction, generating H_2O or H_2O_2 as products. They contain a metal or a Flavin-type coenzyme on the active site (Phale et al. 2019). In addition, it has been shown that some reductases are also involved by some yeast strains. The reductases indicated for the biodegradation of synthetic dyes are Azoreductase, NADH-DCIP reductase, and Malachite green reductase (Danouche et al. 2021b).

Laccase (Lac: EC 1.10.3.2) Synthetic dyes biocatalysis with Lac can be achieved by direct biodegradation of the dye molecule by a nonspecific radical mechanism. This enzymatic pathway has the advantage of avoiding the formation of toxic by-products, such as aromatic amines, which are usually obtained as a result of specific cleavage of the azo bond of various dyes via reductases or chemical catalytic processes (Dave et al. 2015). Several studies have documented the involvement of Lac in the biodegradation of the synthetic dye by yeast strains of *Sterigmatomyces halophilus* SSA-1575 (Al-Tohamy et al. 2020), *Galactomyces geotrichum* GG (Guo et al. 2019), *Cyberlindnera fabianii* (Danouche et al. 2021c), *T. akiyoshidainum* HP2023 (Martorell et al. 2017a), *T. multisporum*, and *T. laibachii* (Pajot et al. 2007).

Tyrosinase (Tyr: E.C. 1.14.18.1): referred also to as monophenol monooxygenase or polyphenol oxidase. It is an oxidase with copper coenzyme, which can be employed for the detoxification of wastewater containing phenol or other organic pollutants (Kim and Uyama 2005). The catalytic reaction of the dyes

with Tyr occurs in two successive steps, a first catalyzing reaction is the o-hydroxylation of the monophenols to the corresponding catechols (monophenolase activity), next a second oxidation of monophenols to the corresponding o-quinones (diphenolase activity) (Duckworth and Coleman 1970). On their involvement in the biodegradation of synthetic dyes by yeast cells, it has been identified in only a few yeast species. (Danouche et al. 2021c) reported their involvement in the biodegradation of the azo dye Acid Red 14 with a yeast strain of *C. fabianii*, in addition to other yeast strains including *G. geotrichum* MTCC (Waghmode et al. 2012a, b), *S. cerevisiae* MTCC 463 (Jadhav et al. 2007), *C. krusei* strains (Charumathi and Das 2011), *Candida* sp. MM 4035, *T. porosum* MM 4037, *C. satwnus* MM 4034, *Barnettozyma californica* MM 4018 (Martorell et al. 2012).

Lignin Peroxidase (LiP: EC 1.11.1.14) LiP is an extracellular enzyme whose enzymatic substrate is nonspecific, this particularity confers it the capacity to degrade various aromatic phenolic and non-phenolic compounds (Chowdhary et al. 2018). For example, the biodegradation of sulfonated azo dye with LiP can be accomplished in two consecutive one-electron oxidations of the oxidized LiP forms by H_2O_2 in the phenolic ring, where the corresponding carbonium ion bearing the azo bond contributes to the formation of quinone and phenyldiazine by nucleophilic attack by H_2O. The phenyldiazine product is then oxidized by O_2 to a phenyl radical and the azo bond is removed as N_2, and then the phenyl radical extracts hydrogen from its surroundings to produce a stable aromatic compound (Chivukula et al. 1995). The catalytic activity of LiP was investigated during the biodegradation of various synthetic dyes by basidiomycota yeast strains of *T. laibachii and T. multisporum* (Pajot et al. 2007), as well as, by ascomycota yeast strains of *S. halophilus* SSA-1575 (Al-Tohamy et al. 2020), *G. geotrichum* (Guo et al. 2019), *P. occidentalis* (Song et al. 2018a), *S. cerevisiae* (Jadhav et al. 2007), *C. krusei* (Charumathi and Das 2011), *Diutina rugosa* (Bankole et al. 2017), and *C. samutprakarnensis* (Song et al. 2018b).

Manganese Peroxidase (EC 1.11.1.13) MnP is a substrate-specific oxidase that oxidizes Mn^{2+} to Mn^{3+} from the surface of the enzyme and subsequently oxidizes phenolic substrates such as model lignin compounds or other organic contaminants (Zhou et al. 2013). Yeast used MnP for the biodegradation of synthetic dyes as well, it was revealed in yeast strains of *C. fabianii* (Danouche et al. 2021c), *P. occidentalis* (Song et al. 2018a), *D. polymorphus, C. tropicalis* (Yang et al. 2008), *T. multisporum,* and *T. laibachii* (Pajot et al. 2007).

Azoreductase (AzoR: EC 1.7.1.6): are a class of enzymes that catalyze the reduction reaction such as the reduction of azo bonds (-N=N-) of azo dyes and nitroaromatic and azoic drugs (Misal and Gawai 2018). The AzoR can be classified according to their structures, or according to flavin dependence. The flavin-dependent class of AzoR can also be divided based on their coenzymes like NADH, NADPH (Saratale et al. 2011; Solís et al. 2012). The involvement of AzoR in dye biodegradation by yeast has been reported in some research employing

yeast strains of *Issatchenkia occidentalis* (Ramalho et al. 2004), *C. fabianii* (Danouche et al. 2021c), *C. krusei* (Charumathi and Das 2011), *S. cerevisiae* MTCC 463 (Jadhav et al. 2007), and *T. beigelii* NCIM-3326 (Saratale et al. 2009a).

NADH-Preferring 2,6-Dichloroindophenol Reductase ***(NADH-DCIP: EC 1.6.99.3)*** NADH-DCIP reductase is an oxidoreductase that reduces 2,6-dichloroindo-phenol (DCIP) with NADH as an electron donor (Nishiya and Yamamoto 2007). Some studies have shown an increase in the NADH-DCIP reductase activity during the biodegradation of various azo dyes with strains of *P. occidentalis* G_1 (Song et al. 2018a), *C. samutprakarnensis* (Song et al. 2018b), *D. rugosa* (Bankole et al. 2017), *P. kudriavzevii* CR-Y103 (Rosu et al. 2018), and *T. beigelii* NCIM-3326 (Saratale et al. 2009a).

Malachite Green Reductase NADH is used as an electron donor by this reductase to transform malachite green into leucomalachite green. It has been first reported by Jadhav and Govindwar (2006) that in *S. cerevisiae* MTCC 463 was used for the biodegradation of green malachite. Next, Jadhav et al. (2008b) demonstrated their implication in the biodegradation of methyl red by *G. geotrichum* MTCC 1360. Also, Charumathi and Das (2011) reported the increase of MG-reductase activity in *C. krusei* used for the biodegradation of Basic Violet 3, as well as, in *S. cerevisiae* used for the biodegradation of Malachite green (Biradar et al. 2016).

Regardless of the mechanisms involved, the performance of the microorganisms in the bioremediation of organic pollutants can be influenced by various environmental factors, including nutrients, pH, temperature, etc. It is therefore critical to emphasize the impact of these parameters on the yeast's ability to eliminate such contaminants.

5.4 Factors Controlling Mycoremediation Performance

Yeast cells are sensitive to the environmental conditions in which they grow. Determining the impact of these physiochemical factors on the efficiency of the removal of synthetic dyes is therefore crucial in order to make the mycoremediation process faster, more efficient, and more practical for large-scale applications. There are two ways to perform this optimization, either using a single-factor optimization approach or based on statistical methods of optimization by the design of the experiment (Gönen and Aksu 2009; Mahmoud 2016).

Carbon and Nitrogen Sources The effect of carbon and nitrogen sources on the bioaccumulation or the biodegradation capacity of dye by yeast strains has been the subject of several studies. It has been reported that at constant sucrose content, the concentrations of both Remazol Black B and Remazol Blue dyes inhibited the growth of *C. tropicalis*, with constant dye concentration, the growth efficiency and the bioaccumulation capacity increased with sucrose concentration up to 15 g L^{-1} (Aksu and Dönmez 2005). This combined effect was also analyzed using

a statistical approach of response surface methodology. Okur et al. (2014) found that the optimal values for dye uptake by *C. tropicalis* correspond to sugar concentration of 5.1 g L^{-1} and a dye concentration of 499 mg L^{-1} of initial dye concentration. Also, Gönen and Aksu (2009) found that the optimum combination predicted by response surface methodology (RSM) confirmed that *C. utilis* was able to bioaccumulate Remazol turquoise blue-G with a maximum uptake yield of 82.0% in 15 g L^{-1} sucrose and 50 mg L^{-1} dye concentration. Additionally, (Das et al. 2010) confirmed that *P. fermentans* MTCC 189 was able to accumulate Basic violet 3 up to 69.8% in 10 mg L^{-1} of dye-containing medium and 24 g L^{-1} sugar extracted from sugarcane bagasse through RSM analysis. Concerning the carbon sources effect on the biodegradation efficiency of dye by yeast cells, it has been reported that the addition of carbon sources, especially glucose at a certain level, stimulates the biodegradation of dyes (Chang et al. 2000; Waghmode et al. 2011). Indeed, glucose is an essential element in several mechanisms, it provides an energy source for yeast growth, it regenerates the redox mediators NADH and FADH, and also it serves as a substrate for the production of H_2O_2, which acts in turn as a co-substrate for MnP and LiP (Swamy and Ramsay 1999; Jafari et al. 2014). In the same way, the supplementation of the medium with nitrogen sources such as peptone, yeast extract, urea, or others favors the regeneration of NADH which is used as an electron donor for the reduction of azo dyes by the different enzymes (Bras et al. 2001).

Dye Concentration The initial dye concentration can significantly influence their removal efficiency. A higher dye concentration gradually decreases the percentage of their decolorization. This can be attributed to the toxicity of the dyes toward the yeast cells, or to a lower biomass production making the decolorization operation inefficient (Saratale et al. 2011). It has been reported by Das et al. (2010) that the bioaccumulation of Acid Blue 93, Direct Red 28, and Basic Violet 3 by *P. fermentans* MTCC 189 decreased as the initial concentration of these dyes increased from 10 to 30 mg L^{-1}. Likewise, Dönmez (2002) and Aksu (2003) described that the increase in the initial dye concentration inhibited the growth and caused a long lag period of *S. cerevisiae and C. tropicalis*. Other studies indicate that the presence of dyes at high concentrations may inhibit azoreductase activity during enzymatic biodegradation of the synthetic dye, due to the binding of dye molecules to the active site of enzymes (Jadhav et al. 2008a; Saratale et al. 2009a).

Temperature Temperature is one of the crucial factors that influence the metabolic pathways, the enzymatic activity, and the physicochemical interaction of dye molecules with the cell wall. According to the available literature, no study has been devoted to the question of the effect of temperature on the bioaccumulation of dyes using yeast cells. Therefore, it is very important to consider this factor as a research question for future studies. Regarding the effect of temperature on the ability of yeast to biodegrade dyes, many studies have been conducted on the activation energy of the involved enzymes (Chequer et al. 2013; Miranda et al. 2013). The performance of decolorization increased with increasing temperature to the optimum temperature, then a reduction in activity occurs at higher temperatures (Tan et al. 2013, 2014, 2016). The decrease in the ability of yeast strains to remove the dye molecules can be

attributed to the denaturation of the enzymes involved or to the resulting loss of cell viability (Saratale et al. 2009b).

pH pH of solutions is one of the most influential factors on the ability of yeast cells to bioremediate various organic pollutants, including synthetic dyes, it can modify the physicochemical properties of the dye molecules, as well as the physicochemical properties of the yeast surface where the cell-dye molecule interaction initially occurs (Fu and Viraraghavan 2002). At low pH, the surface of yeast cells becomes protonated with a positive charge, which promotes the binding of anionic dyes. On the other hand, at higher pH values, the cell surface acquires a negative charge, resulting in the electrostatic attraction of cationic dyes (Charumathi and Das 2012). The pH may have an inhibitory effect on the transport of dye molecules across the cell membrane, which is considered the first stage of the intracellular biodegradation or bioaccumulation process (Khan et al. 2013). In addition, the initial pH can affect the physiology of the yeast cells and the enzymatic activity. Most yeast strains show better decolorization efficiency under neutral or acidic conditions. The optimal value for the bioaccumulation of Remazol Blue, Reactive Red, and Reactive Black by the yeast strain *C. tropicalis* (Dönmez and Aksu 2002) as well as for Remazol Red RB, Remazol Black B, and Remazol Blue by *S. Cerevisiae* (Aksu 2003) was observed was 3.0. Likewise, Das et al. (2010) investigated that the maximum bioaccumulation rate of Basic Violet3, Direct Red 28, and Acid Blue 93 by growing cells of *P. fermentans* MTCC 189 was at pH 5.0.

Shaking Agitation allows a uniform distribution of oxygen and nutrients in the medium. It also facilitates the exchange of gases produced during the fermentation process of dyes by yeast cells (Yu and Wen 2005; Pajot et al. 2007; Yang et al. 2008; Martorell et al. 2017b). Differing opinions have been expressed regarding the effect of oxygen on the decolorization process, with some researchers considering that during the dye reduction reaction by the reductase, oxygen behaves as a stronger electron acceptor than the dye molecule, thus preventing the azo dye reduction reaction (Kalyani et al. 2008). On the other hand, other research indicates that oxidizing enzymes, such as laccases, require oxygen to oxidize aromatic molecules, including dyes (Thurston 1994). Therefore, it is necessary to optimize the level of agitation that regulates the concentration of dissolved oxygen in the medium throughout the yeast decolorization process to ensure an effective treatment.

5.5 Conclusions

On the basis of the research discussed in this chapter, it is appropriate to consider mycoremediation as a cost-effective, eco-friendly, and efficient approach for the biodegradation of organic contaminants. However, an efficient mycoremediation process requires the optimization of physicochemical conditions, notably the initial concentration of the pollutant, the supply of nitrogen or carbon sources, as well as growth conditions such as pH, temperature, and agitation. On the other hand, the

effectiveness of this kind of bio-processes should not be limited to emphasizing the degradation of the considered pollutant molecule; instead, it requires characterization and evaluation of the toxicity of the obtained by-products, since they can have more harmful effects than the original molecule. Lastly, we strongly recommend the application of this emerging biotechnology in wastewater treatment at pilot or large scale in order to prove its potential application.

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