Review

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Biodegradation of atrazine, a review of its metabolic pathways

https://doi.org/10.1515/ijcre-2024-0241
Received August 15, 2024; accepted February 14, 2025;
published online March 4, 2025

Abstract: Atrazine is a triazine organochlorine herbicide used commonly in some countries like Mexico, however it is hazardous for human, vegetable and animal life. Atrazine reaches groundwater drinking sources, causing serious illnesses in the population. Understanding the mineralized atrazine biodegradation process is a crucial issue in dealing with soils containing atrazine. Bioremediation of soil and water contamination involves a complex interplay between mass transport and biological processes. Soil adsorption, solubility, and interfacial transport limit the availability of contaminants to microorganisms. This review summarizes the recent studies about metabolic pathways and enzymes to degrade atrazine to carbon dioxide, ammonia, water and biomass carried out by some bacteria and fungi species. The biodegradation of atrazine was analyzed, considering the different metabolic pathways of bacteria and fungi. Biodegradation of atrazine by bacteria is well studied, but the fungal metabolism of this compound remains less clear. Some species of white-rot fungi can naturally biodegrade complex compounds, such as lignin, an aromatic polymer that is a major component of plant cell walls. The lignin has a polyphenolic structure that presents similarities with many aromatic pollutants and herbicides like atrazine. A deep understanding of the mechanisms involved is a successful tool for the design of new strategies for biodegrading. A comparison of the rates of degradation between physicochemical and biological processes is included, and some recommendations for the microorganism species and conditions.

Keywords: atrazine; biodegradation; metabolic pathway; rot white fungi; aerobic bacteria

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1 Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-1,3,5-tria zine) is a triazine organochlorine herbicide used to control wild plants in extensive crops. It is considered a moderately mobile and leachable substance in soil, which makes it an important factor of the contamination of surface and groundwater. It has a half-life of 100 days in water; and from 30 to 400 days in soil, however, it has an extended half-life in air, and it is persistent. Therefore, atrazine may enter at food chain, affecting biodiversity and human health [1].

Atrazine was invented and patented in 1958 in Switzerland. Since then, it has been utilized as a pre- and post-emergent herbicide. It is recognized as a systemic herbicide since it is absorbed by roots. It has great efficacy against a wide spectrum of weeds, it has a stable molecular structure, a good cost-effectiveness index, and the ability to mitigate soil erosion. It has significant mobility, limited volatilization, resistance to aerobic degradation, and resistance to abiotic hydrolysis. These characteristics contribute to its heightened accumulation in soil and subsequently contamination of both ground and surface water sources. Atrazine and its dealkilated and deaminated metabolites are toxic, carcinogenic, and endocrine disruptors too [2], [3].

This herbicide is formed by a heterocyclic aromatic ring alternating carbon and nitrogen atoms, besides chlorine substituent. Atrazine's chemical formula is $C_8H_{14}N_5Cl$. Figure 1 shows its molecular structure.

It is very toxic for aquatic life, and it is bioaccumulated by fish, Phyto and zoo plankton, microcrustaceans, mollusks, amphibians, bees, birds, and mammals. Atrazine affects the carbohydrates and lipids metabolism in humans [4].

Atrazine poison in humans affects cholinesterase activity and produces disorders in the central nervous system, cramping in the abdomen, tightness in the chest, dizziness headache, diarrhea, hypotension and psychological complications, peripheral nerve damage, decreased performance in psychological tests, sensitivity to specific chemicals [5].

The Environmental Protection Agency (EPA) classifies atrazine as belonging to toxicity class III; the US Environmental Protection Agency classified atrazine as an herbicide that disrupts the endocrine system; and it has been classified as a carcinogenic herbicide by the International Agency for Research on Cancer [6]. In the European Union, the maximum value allowed in water for human consumption is 0.1 μg/L for all agrotoxic compounds [2].

Atrazine use is prohibited in 44 countries like: all European Union countries (since 1992), the United Kingdom, Uruguay, Cambodia, Egypt, Gambia, Morocco, Niger, Palestina, Senegal, Switzerland, United Arab Emirates, Türkiye, and others. The main reason is because it is a public and environmental health problem, without there being a way to contain contamination of drinking water. In Argentina there is an initiative for the cancellation of all authorized brand registrations and consequently the use of the active ingredient atrazine, given the risk of serious and irreparable damage to the environment, biodiversity, and public health that atrazine represents in agriculture and agri-food [4]. Unfortunately, in Mexico, as in many other countries, there are no regulations regarding the use of atrazine.

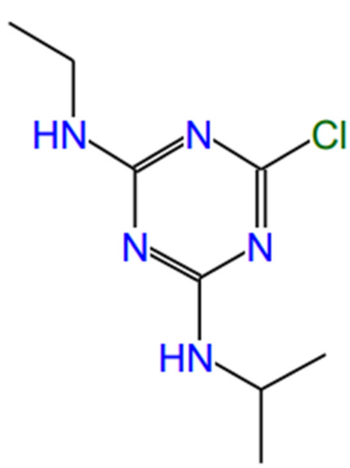


Figure 1: Molecular structure of atrazine.

However, the presence of atrazine has been reported in drinking water and in raw milk at United States and European Union, at levels exceeding their maximum permitted levels. Atrazine's maximum level in the United States is 3 μg/L, and in the European Union, it is 0.1 μg/L [2].

There are physical, chemical, and biological mechanisms for the degradation of the atrazine. Some physical methods include the adsorption of atrazine in porous materials. Chemical methods consist of oxidation reactions using oxidation reactions like ozone. Biodegradation could be carried out by vegetables or microorganisms that have enzymatic systems to oxidize atrazine.

The bioremediation of soil and water contamination involves a complex interplay between mass transport and biological processes. Soil adsorption, solubility, and interfacial transport limit the availability of contaminants to degrade microorganisms.

Some intermediates and residues of atrazine degradation have the capacity to endure in agricultural fields and surface water for long periods [7], [8], contaminating surface and water bodies, and crop fields. The main intermediate metabolites of atrazine are De-ethyl-atrazine (DEA), De-isopropyl-atrazine (DEIA), De-ethyl-de-isopropyl-atrazine (DEDIA), Hydroxy-atrazine (HyA), Diamino-chlorotriazine (DACT), 1-hydroxy-isopropyl-atrazine (HIATZ), Dialkylated atrazine (DIDEA), Mercapturic acid, and Cyanuric acid [4]. It is important to mention that the toxicity of some of those metabolites is greater than the atrazine itself. Atrazine, DEA, DEIA, and DEDIA share a mechanism of toxicity on the endocrine system, according to the United States Environmental Protection Agency (USEPA).

Although this work aims to describe the microbiological methods of degradation, it includes a brief description of the physical, chemical, and biological methods.

2 Physical degradation

Some physical methods to remove atrazine from a liquid stream include activated carbon, biochar, graphene, and carbon nanotubes adsorption. All of them have a porous structure with a large specific surface area and some other substances like organic –OH, $-CH_3$, $-CH_2$, that engage the physisorption process when atrazine is in aqueous solutions [9].

Biochar is produced by biomass pyrolysis under oxygen limited conditions; it is a potent adsorption for insecticides, synthetic biphenyls, polymer of aromatic hydrocarbons, as well as atrazine [10], [11]. Modifications to biochar, such as incorporate manganese or iron, increase its surface area and introduce more functional groups, improving its atrazine adsorption capacity [11].

Atrazine adsorption onto carbonaceous adsorbents involves multiple mechanisms, such as hydrogen bonding, van der Waals forces, hydrophobic, and electrostatic interactions, partition coefficients, pore size, and π–π electron donor-acceptor interactions. Atrazine is a hydrophobic molecule and can be adsorbed with the aromatic rings in the activated carbon or biochar structure through electron donor-acceptor interactions; it can interact through electrostatics interactions (attractions or repulsions) as a function of pH level.

The highest atrazine adsorption capacity of activated carbon was found between 13.95 and 712.1 mg/g, biochar capacity between 4.55 and 409.84 mg/g, and carbon nanotubes between 28.21 and 110.8 mg/g [11]–[13]. This is a good option to eliminate atrazine contained in water or other liquids; however, it is not a form to degrade because atrazine molecules are adsorbed, but it was necessary to desorb it on time to reuse the material again.

3 Chemical degradation

The main chemical methods for degrading atrazine are the Fenton reaction, ozone oxidation, electrochemical degradation, sulfate radical (SO_4^{-}) oxidation, and photocatalysis, all of which consist of the oxidative destruction of the organic molecule.

Fenton method uses a mixture of hydrogen peroxide (H_2O_2) and iron ions (Fe^{2+}) salts to generate hydroxyl radicals ($^{\cdot}OH$), which are highly reactive and can degrade atrazine:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + {}^{\cdot}OH \quad (1)$$

The hydroxyl radicals attack atrazine to break it down into smaller compounds like urea, formic and oxalic acids:

$$C_8H_{14}ClN_5 + {}^{\cdot}OH \rightarrow C_8H_{13}ClN_5^{\cdot} + H_2O \quad (2)$$

As can be seen, a new radical is formed, and it can react with another hydroxyl radical:

$$C_8H_{13}ClN_5 + {}^{\cdot}OH \rightarrow C_8H_{12}ClN_5^{\cdot} + H_2O \quad (3)$$

Oxidation reactions continue to obtain organic acids, carbon dioxide, water, and cyanuric acid. Nevertheless, cyanuric acid is a toxic molecule too. To avoid cyanuric acid remaining in water it is necessary to ensure that the oxidation process is exhausted.

Ozone (O_3) molecules produce hydroxyl radicals and oxygen in the presence of water too:

$$O_3 + H_2O \rightarrow 2{}^{\cdot}OH + O_2 \quad (4)$$

Then, hydroxyl radicals react with atrazine with the same mechanism shown before. Ozone oxidation is considered one of the powerful oxidants, though the high prices of ozone generation limit its practical implementation [12].

Electrochemical degradation involves electrical energy to generate reactive species to break down atrazine molecules. It can involve:

(a) The generation of hydroxyl radicals ($^{\cdot}OH$) from water:

$$H_2O \rightarrow {}^{\cdot}OH + H^{+} + e^{-} \quad (5)$$

(b) The free chlorine (Cl_2) generation and then the activation by UV light irradiation to produce hydroxyl ($^{\cdot}OH$), and chlorine ($Cl^{\cdot}$) radicals:

$$Cl_2 + H_2O \rightarrow HOCl + H^{+} + Cl^{-} \quad (6)$$

$$HOCl \rightarrow {}^{\cdot}OH + Cl^{\cdot} \quad (7)$$

Again, radicals can attack atrazine molecules. This method is quicker than Fenton [14], [15].

(c) The combination of electrochemical generation of hydrogen peroxide (H_2O_2) with iron ions (Fe^{2+}) to produce hydroxyl radicals:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + {}^{\cdot}OH \quad (8)$$

Electrochemical processes have high efficiency, are versatile can be applied to degrade other recalcitrant compounds, and can be precisely controlled. However, they are energy consumption, electrodes require maintenance, their initial costs are high, sometimes they produce toxic byproducts, and they have operational complexity [16].

The sulfate radical oxidation takes place when persulfate or peroxy-mono-sulfate are activated by heat light or metal ions to produce super oxidative sulfate radicals (SO_4^{-}) which oxides atrazine [14]. Sulfate radicals can be generated through:

(a) Thermal or UV activation: heating persulfate ($S_2O_8^{2-}$) to obtain sulfate radicals:

$$S_2O_8^{2-} \rightarrow 2SO_{4-}^{\cdot} \quad (9)$$

(b) Peroxymonosulfate activation:

$$HSO_{5-} + Fe^{2+} \rightarrow SO_{4-}^{\cdot} + Fe^{3+} + OH^{-} \quad (10)$$

Once sulfate radicals are generated, they react with atrazine in two paths:

– Sulfate radicals can abstract hydrogen atoms from atrazine, to produce intermediate radicals:

$$C_8H_{14}ClN_5 + SO_{4-}^{\cdot} \rightarrow C_8H_{13}ClN_5^{\cdot} + HSO_{4-} \quad (11)$$

– Or sulfate radicals oxidize atrazine by electron transfer:

$$C_8H_{14}ClN_5 + SO^{\cdot}_{4-} \rightarrow C_8H_{14}ClN_{5+} + SO_4^{2-} \quad (12)$$

Sulfate oxidation produces cyanuric acid as a byproduct, in the same way the other oxidation methods [15].

Another interesting method is photocatalysis using titanium oxide with a pore size of 25 μm (TiO_2 P25), and TiO_2 modified with carbon catalyst in a tubular photochemical reactor equipped with a compound parabolic collector irradiated by solar light, in aqueous systems. In the first step titanium oxide is activated by UV light, producing electron pairs:

$$TiO_2 + UV \rightarrow TiO_2\left(e^- + h^+\right) \quad (13)$$

Then, protons oxidize water into hydroxyl radicals ($^{\cdot}OH$), and electrons (e^-) reduce oxygen to form superoxide radicals ($O_2^{\ -}$):

$$h^+ + H_2O \rightarrow {}^{\cdot}OH + H^+ \quad (14)$$

$$e^- + O_{2-} \rightarrow O^{\cdot}_{2-} \quad (15)$$

Hydroxyl radicals react with atrazine molecules in the same way shown before [15], [17].

Temperature, pH level, and initial atrazine concentration affect all chemical and physical degradation [6]. Temperature enhanced atrazine solubility, making its hydrolysis; increasing temperature the kinetics constants of all reactions rise (almost all reactions are irreversible) [18].

The level of pH affects hydrolysis atrazine reactions, usually acid or basic conditions increase degradation because acid media can protonate atrazine, making it more susceptible to nucleophilic attack; basic media can enhance the nucleophilicity of water. On the other hand, the atrazine degradation with UV light and hydrogen peroxide is higher in acid conditions, because hydrogen peroxide is more stable and produces more hydroxyl radicals. Besides, ozone oxidation is more effective in alkaline conditions.

Physics and chemical degradation methods are useful if atrazine is dissolved in a liquid phase, like water [19], [20].

4 Biological degradation

Some vegetable species have been reported by their capacity to degrade atrazine, such as *Myriophyllum spicatum* (Haloragaceae) and *Lolium mulfiflorum* (Poaceae). *M. spicatum* is an aquatic herb of the *Haloragaceae* family, it was proven to eliminate atrazine from a lake sediment in China. This herb is able to germinate and grow in the presence of 1 mg of atrazine by 1 kg of soil, and it presented a 10 % higher atrazine removal capacity than natural attenuation. Although they obtained good results, it is not clear if *M. spicatum* biodegraded atrazine, or if atrazine was accumulated in leaves and stems, or if bacteria growing on the roots were responsible for biodegrading [21]. *M. spicatum* original distribution was in Europe, Asia, and North Africa, but unfortunately is highly invasive and has extended worldwide.

L. mulfiflorum is an herbaceous annual ryegrass presumably native to Europe. It can grow in template climates, and can be used as an emergence forage, and as a cover crop in winter and ornamentally. However, it can become a weed and highly invasive species. It has been reported that can survive on atrazine concentrations between 1 and 10 mg kg^{-1} of soil. The presence of this herb contributes to diminishing atrazine concentrations in soils [22]. However, as in the *M. spicatum* study, it is not clear if atrazine is accumulated in the herb if is degraded by bacteria in the root or accumulates in the plant. The main problem of atrazine accumulation in plants is that those plants can be eaten by animals, and then atrazine scales up in the food chain.

Removal of pollutants to a less toxic form in soil is done mainly by microorganisms [23]. Microbiological degradation of atrazine can take place by bacterial, fungus, and yeast strains; it is increasingly being recognized as an eco-friendly, economically feasible, and sustainable bioremediation process [24]. The factors that mainly affect micro biodegradation are pH, temperature, soil moisture, microbial activity, presence of different contaminants, initial concentration of the contaminant, and the species present in the soil or bioreactor where biodegrading takes place [23]. Usually, increasing temperature and humidity increases the biodegrading rate. On the other hand, optimal pH level, and nutritional requirements depend on the microorganism species used [25].

Bioremediation with bacteria strains treatment includes N-dealkylation, dichlorination, and ring cleavage [26]. Atrazine biodegradation can be initiated by N-dealkylation of the ethyl group or isopropyl chain to produce deethylatrazine (DEA) or deisopropylatrazine (DEIA) [4], [14], [16], [27], [28].

Biodegradation of atrazine by bacteria is well studied, but the fungal metabolism of this compound remains less clear [29]. Fungus can biodegrade complex compounds in a natural way, like lignin, they are in charge of the biodegradation of dead trees and all kinds of vegetation. Lignin is a polyphenolic structure that presents structure similarities with many aromatic pollutants [30].

Some fungus strains degrade atrazine, because they produce extracellular enzymes such as laccases, peroxidases, and some other ligninolytic enzymes [31]. White-rot fungi are the main producers of nonspecific oxidases, then fungi are considered most effective in bioremediation, as a method to convert toxic, and recalcitrant pollutants in water and soil, into environmentally benign products for the action of biological treatments [32]. Laccases, lignin peroxidases, and manganese peroxidases are responsible for the degradation of the most recalcitrant molecules in nature [33]. Furthermore, white-rot fungi tolerate extreme conditions, such as water deficit and nonsterile medium.

On the other hand, yeasts and molds can utilize aromatic compounds as a growth substrate and convert it into biomass, carbon dioxide, and water. Even though, they have the ability to cometabolize halogenated and nitro aromatics, in the presence of aromatics, to their corresponding catechol or halogenated muconic acids, however, they cannot be further degraded and remains as end products [34]. This is because they cleave aromatic rings exclusively via the ortho pathway [35]. Some yeast species can degrade atrazine, such as *Pichia kudriavzevii* [36], *Cryptococcus laurentii* [37], *Sacharomyces cerevisiae* [38].

Biodegradation *in situ* of atrazine and other herbicides with similar chemical structures is an important issue to prevent further water source contamination and that they enter into the food chain. Applying the knowledge developed about the metabolic pathways involved in biodegradation may help to solve problems related to herbicide contamination in water and soils.

The objective of this work is to compile microbiological methods for mineralizing atrazine into carbon dioxide and water, specifically for bacteria and fungi. This study can help to improve methodologies for biodegradation *in situ* or bioreactors. The study of the metabolic pathways involved, the enzymes, co-enzymes, and co-factors that catalyze each bioreaction, gives some proposals and keys to improve it. This review aims to help improve the implementation of methods for atrazine degradation by microorganisms and provides a brief review of the available physicochemical methods.

5 Bacteria's metabolic pathways

Bacteria that produce enzymes such as hydroxiatrazine hydrolase, atrazine chlorohydrolase, allophanate amidohydrolase, cyanuric acid amidohydrolase, and some others, can break down atrazine molecules. Some reported bacteria able to degrade atrazine are *Pseudomonas strain ADP*, *Acinetobacter lwoffii DNS*, *Alcaligenes*, *Klebsiella*, *Agrobacterium*, *Acinetobacter*, *Rhodococcus*, *Clavibacter*, *Streptomyces*, *Bacillus*, *Pseudomonas*, *and Arthrobacter*. However, only *Arthrobacter sp.*, *Pseudomonas sp.*, and *Bacillus sp*. have been reported to be capable of converting atrazine into biomass, CO_2, H_2O, and N_2. Other bacteria species do not produce the enzymes downstream in the metabolic pathway to degrade atrazine, such as allophanate amidohydrolase or cyanuric acid amidohydrolase, then, sometimes they can produce more toxic molecules than atrazine. *Arthrobacter sp.*, *Pseudomonas sp.*, and *Bacillus sp*. use atrazine as carbon and nitrogen sources [39]. Better biodegradation rates were reported using mixing of two or more bacteria strains [40]–[43].

The metabolic pathway to degrade atrazine to cyanuric acid has been studied in detail for *Pseudomonas sp*. Conversion of atrazine to cyanuric acid involves eight different genes named: *atzA, atzB, atzC, atzD, atzE, atzF, trzN* and *trzD* [44].

For study reasons, the metabolic pathway can be divided into two stages: (1) Conversion of atrazine to cyanuric acid, and (2) Mineralization of cyanuric acid to carbon dioxide and molecular nitrogen (N_2). Stage one in which the atrazine is catabolized to cyanuric acid is made in three enzymatic steps, see Figure 2. Stage two consists in the further conversion of cyanuric acid into ammonia and carbon dioxide, by ring cleavage.

In the first reaction of Stage 1 atrazine is converted to hydroxy atrazine and HCl, this reaction can be catalyzed by two enzymes, triazine hydrolase (trzN), encoded by gene *Pa-trzN*, and atrazine chloro-hydrolase (*atzA*) encoded by gene *Ps-atzA G-124*. This reaction requires a water molecule,

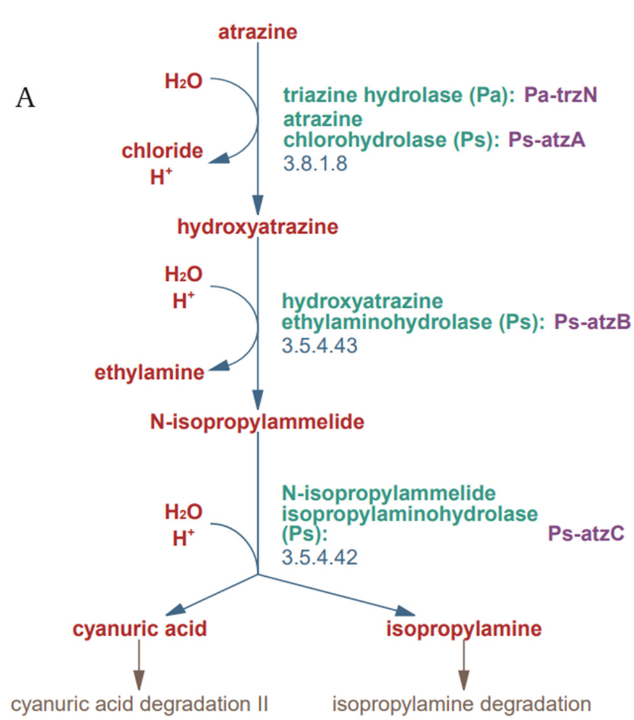


Figure 2: Stage one on the metabolic pathway of atrazine biodegradation to cyanuric acid and isopropylamine. https://metacyc.org/pathway?orgid=META&id=P141-PWY&detail-level=4.

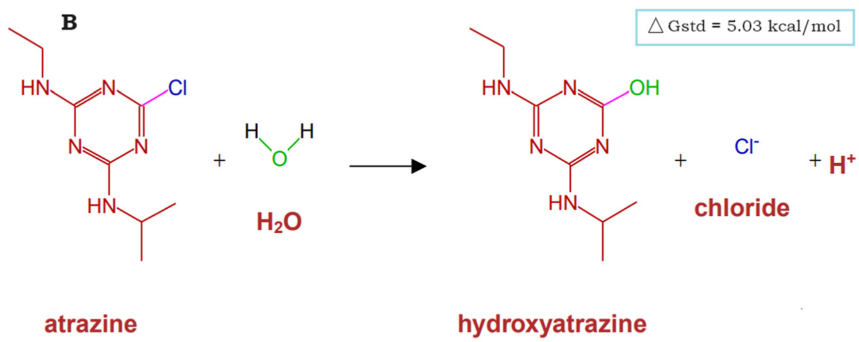


Figure 3: The first reaction of stage one is atrazine to hydroxyatrazine bioreaction. https://metacyc.org/pathway?orgid=META&id=P141-PWY&detail-level=4.

produces one H^+, and needs 5.03 kcal/mol, see Figure 3. Enzyme triazine hydrolase is found in Gram-positive bacteria, it has a very wide substrate specificity [45], then it can convert dozens of s-triazine ring compounds into cyanuric acid [46]. On the other side, atrazine chloro-hydrolase is found in Gram-negative bacteria, it has a much narrower specificity [31]. Therefore, gram-positive and gram-negative bacteria can carry out the degradation of atrazine to hydroxyatrazine, however, if it is necessary to degrade only atrazine it could be better to use Gramm negative bacteria. On the other hand, if there is an herbicide mix it is better to use Gramm-positive bacteria such as *Arthrobacter sp.*, *Pseudomonas sp.*, or *Bacillus sp.* It is essential to consider that Gramm-negative bacteria do not have the enzymes for degrading cyanuric acid.

It is important to mention that staying in an atmosphere with more than 90 ppm or consumption of 30 mg of cyanuric acid provokes severe poisoning. Staying in an atmosphere containing 300 ppm or consuming between 50 and 300 mg of cyanuric acid cause death in a few minutes.

In the second reaction of Stage 1, hydroxyatrazine is converted to n-isopropylammelide by the enzyme hydroxyatrazine ethylaminohydrolase (atzB), see Figure 4. This reaction requires one molecule of water, one H^+, and 7.97 kcal/mol. It produces a molecule of ethylamine, then two carbons and one nitrogen are separated from the original atrazine molecule.

Reaction 3 of stage one consists of the conversion of n-isopropylammelide to cyanuric acid and isopropyl amine, it is catalyzed by the enzyme n-isopropyl ammelide isopropylaminohydrolase (Ps), gene atzC G-115 encodes that, see Figure 5. For this reaction is necessary to spend an H^+, a molecule of water, and 7.97 kcal/mol, also it is produced a molecule of isopropyl amine, so that, three carbons and one nitrogen atom are separated from the atrazine original molecule [32], [33], [36]–[38], [47].

All reactions in this first stage of the metabolic pathway are energy consumption, their Gibbs energy consumption is: 5.03, 7.97, and 7.97 kcal/mol respectively, and their net energy consumption is equal to 20.97 kcal/mol. This is the reason this metabolic pathway (stage one) takes place only under aerobic conditions. Cells consume three ATP molecules to produce three ADP molecules, at this point of the metabolic pathway, because for each phosphate bond broken of ATP cells obtain 7.1 kcal/mol.

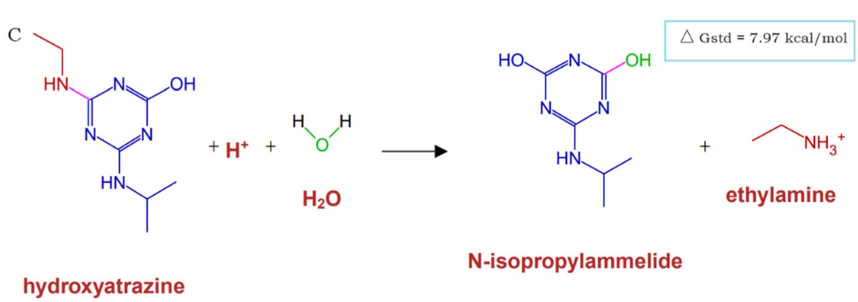


Figure 4: Second reaction of stage one, hydroxyatrazine to n-isopropylammelide bioreaction. https://metacyc.org/pathway?orgid=META&id=P141-PWY&detail-level=4.

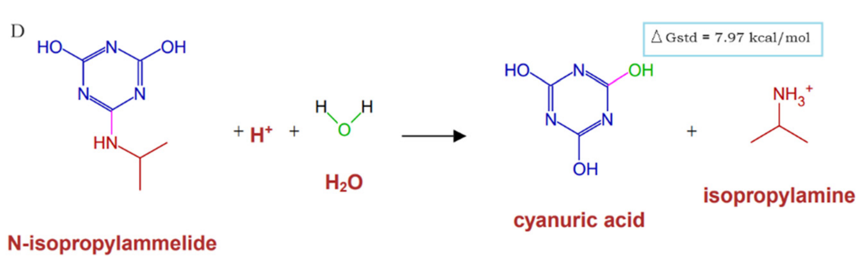


Figure 5: The third reaction of stage one is the bioreaction of n-isopropylammelide to cyanuric acid and isopropylamine. https://metacyc.org/pathway?orgid=META&id=P141-PWY&detail-level=4.

Figure 6 shows the second stage of atrazine biodegradation (sequence of reactions to cyanuric acid to carbon dioxide, nitrogen, and water), where cyanuric acid is mineralized. This stage has three enzymatic reactions and two spontaneous reactions. Once the atrazine molecule has been converted into cyanuric acid, it is converted into 1-carboxybiuret by the enzyme cyanuric acid amidohydrolase (AtzD). A water molecule is consumed, and 20.14 kcal/mol and a H+ are produced, see Figure 7. This is the first reaction where cells obtain energy from atrazine molecules.

After, 1-carboxybiuret is converted into biuret spontaneously, as can be seen in Figure 8. This reactor needs an H^+, and releases 7.46 kcal/mol, and a molecule of carbon dioxide.

Next, a biuret with a molecule of water is transformed into urea-1-caroxylate plus an ammonium molecule, by biuret amidohydrolase (Hh), as it is shown in Figure 9. This reaction consumes a molecule of water and releases a molecule of ammonium and 5.34 kcal/mol.

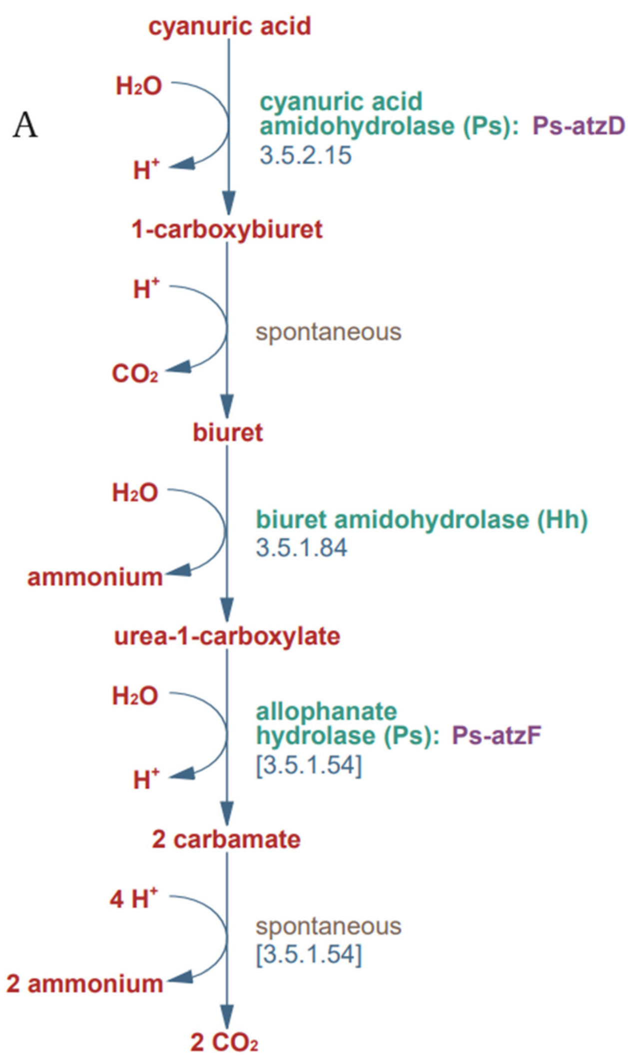


Figure 6: Mineralization of cyanuric acid. https://metacyc.org/pathway?orgid=META&id=PWY-5724&detail-level=4.

The next step consists of the degradation of urea-1-carboxylate into 2 molecules of carbamate by the allophanate hydrolase enzyme (AtzF). This reaction releases much more energy than the other reactions of the second stage, 97.06 kcal/mol, see Figure 10.

Finally, each mole of carbamate broke into two ammonium and two carbon dioxide molecules [41], as can be seen in Figure 11. This reaction is slightly endothermic because it consumes 3.82 kcal/mol.

The enzyme AtzD has activity only if cyanuric acid and methyl-isocyanuric acid are in culture media [49], moreover, enzyme TrzD is not active with any substrate than cyanuric acid [50]. AtzD acts with AtzE and AtzF to hydrolyze cyanuric acid to yield 3 mol of carbon dioxide and ammonia by mole of cyanuric acid [48].

It is important to know the general energy balance, to understand if atrazine can be a source of carbon, energy, and nitrogen source for cells. Although cells have all the enzymes required to break and mineralize atrazine molecules, they require energy to use atrazine as the sole support for life. All bioreactions with positive Gibbs energy are endothermic, they require energy; then cells need to spend ATP to make the reactions.

Cells always live near standard conditions (25 °C, and 1 atm), for example, *Arthrobacter sp.* grows at temperatures between 25 and 45 °C, and its optimal growth temperatures are between 37 and 41 °C; *Pseudomonas sp*. can grow at temperatures between 4 and 42 °C, with an optimal growth over 20 °C; *Bacillus sp*. has an optimal growth temperature between 28 and 35 °C.

Reactions inside cells produce chemical energy in the forms of ATP, NADH, and NADPH, but normally they generate too little heat compared with energy reactions. Then the entropy changes are negligible ($\Delta \boldsymbol{S} \sim \boldsymbol{0}$), considering the Gibbs energy change ($\Delta \boldsymbol{G}$) equation, enthalpy changes are equal to energy Gibbs changes:

$$\Delta G = \Delta H - T\,\Delta S \tag{16}$$

The first four reactions of stage two release energy (130 kcal/mol), however, the last one requires a minimum amount of energy, 3.82 kcal/mol. The net energy production of stage two is equal to 126.18 kcal/mol.

Fungi can use a wide range of carbon sources, and they have many metabolic pathways for it. Those metabolic pathways are regulated to allow the preferential utilization of the most energetically favorable compounds, in the first place glucose. However, in the absence of the easier carbon source compounds, the regulation of the carbon metabolism system represses some pathways and activates others, such

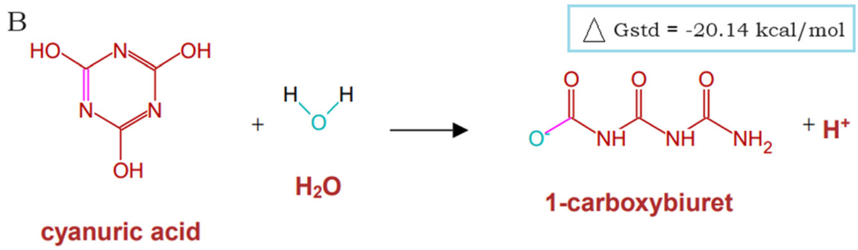


Figure 7: Bioreaction of cyanuric acid to 1-carboxybiuret. https://metacyc.org/pathway?orgid=META&id=PWY-5724&detail-level=4.

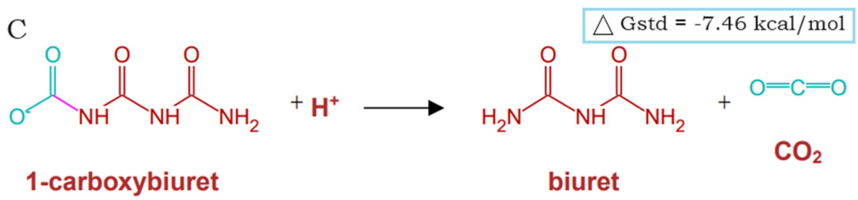


Figure 8: Spontaneous reaction to convert 1-carboxybiuret into biuret. https://metacyc.org/pathway?orgid=META&id=PWY-5724&detail-level=4.

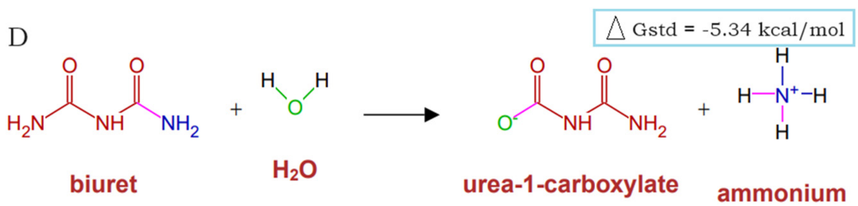


Figure 9: Conversion of biuret into urea-1carboxylate and ammonium. https://metacyc.org/pathway?orgid=META&id=PWY-5724&detail-level=4.

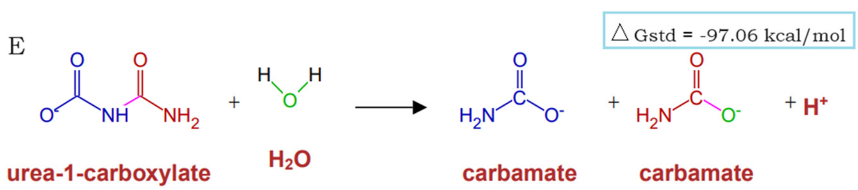


Figure 10: Catabolism of urea 1-carboxylate to carbamate. https://metacyc.org/pathway?orgid=META&id=PWY-5724&detail-level=4.

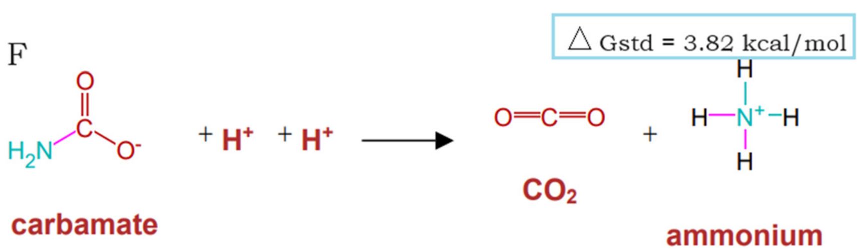


Figure 11: Degradation of carbamate to carbon dioxide and ammonium. https://metacyc.org/pathway?orgid=META&id=PWY-5724&detail-level=4.

as the one shown before. In order to use atrazine as a nitrogen source, fungi need to produce glutamate or glutamine, both of which can be constructed from the ammonium [49] obtained in the last reaction of the metabolic pathway, shown in Figure 11.

The global energy balance of atrazine biodegradation metabolic pathway for *Pseudomonas sp.* (stages 1 and 2) releases 105.21 kcal/mol of atrazine. Then it can be supposed that atrazine can be an energy, carbon, and nitrogen source. However, many authors have reported that most bacteria can grow on atrazine and s-triazines as the sole nitrogen source, but will no grow on them as the sole carbon source [26], [28], [38], [50]–[52]. Probably this is because the first stage of the metabolic pathway requires energy, and only after cyanuric acid has been obtained cells are able to obtain carbon and energy. Another possibility is the lack of enzymes needed in the second stage, to break cyanuric acid molecules.

Summarizing all metabolic pathways, six molecules of water are required for each atrazine molecule, and there are

Table 1: Enzymes catalyze atrazine metabolic pathway and the genes that codified them. https://metacyc.org/pathway?orgid=META&id=PWY-5724&detail-level=4.

Enzyme ID	Enzyme name	Gene	Specie
trzN	Triazine hydrolase atrazine	*G-10313*	*Paenarthrobacter aurescens*
atzA	Atrazine chlorohydrolase	*G-124*	*Pseudomonas sp*
atzB	Hydroxyatrazine ethylaminohydrolase	*G-114*	*Pseudomonas sp*
atzC	N-isoproylammelide isopropylaminohydrolase	*G-115*	*Pseudomonas sp*
atzD	Cyanuric acid amidohydrolase	*G-10314*	*Pseudomonas sp*
Hh	Biuret amidohydrolase	N D	*Herbaspirillum huttiense*
atzF	Allophanate hydrolase	*G-10288*	*Pseudomonas sp*

produced one of propylamine, one of ethylamine, one of hydrochloric acid, a three of ammonium, and three of carbon dioxide:

$$C_8H_{14}N_5Cl + 6H_2O \rightarrow C_3H_9N + C_2H_7N + 3NH_3 + HCl + 3CO_2 \quad (17)$$

There are differences in organization and regulation between genes encoding the broad and narrow specificity enzymes [46]. Narrow specificity enzymes are encoded by the *atzD G-10314, and atzF G-10288* genes, which are contiguous on plasmid *pADP-1* and are coregulated under the control of the upstream *atzR* gene product. Expression of *atzD, E, and F* genes is controlled by nitrogen limitation and the presence of cyanuric acid [53]. Broad specificity genes show a different behavior, they are not continuous on plasmid *pADP-1*. Table 1 shows enzymes that catalyze the atrazine metabolic pathway and the genes that codify them.

Figure 12 shows atrazine metabolic pathways carried out by *Rhodococcus sp TE1*, isolated from contaminated soil with s-ethyl-N-dipropylthio-carbamate. This bacterium is Gram-positive and can degrade atrazine to deethyl-atrazine and deethylsimazime, in a ratio of about 3:1. However these compounds are not degraded further, and they are accumulated in the growth medium [39].

Both reactions in this pathway are shown in detail in Figure 13, they require two reduced electron carriers and produce an oxidized electron carrier. An electron carrier is a chemical compound that can accept or donate electrons from/to another chemical compound. Reduced electron carriers are molecules that have gained electrons through a reduction. These carriers are indispensable in cellular respiration and photosynthesis; the most common reduced electron carriers are NADH and $FADH_2$ (produced in some metabolic pathways such as glycolysis and Krebs cycle).

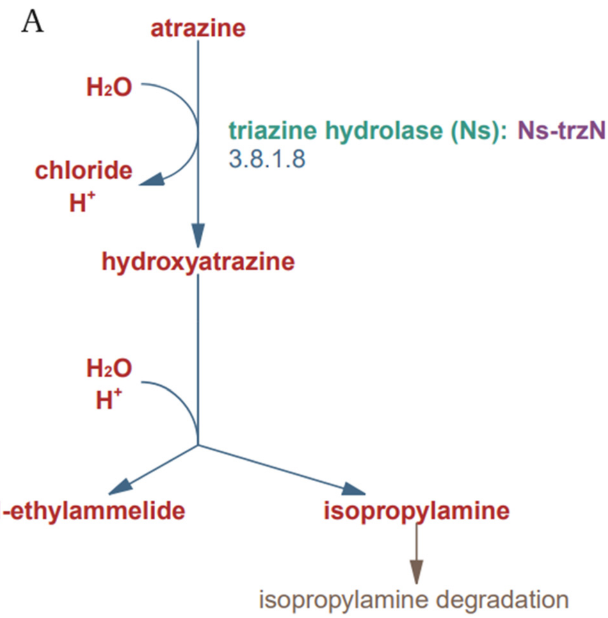


Figure 12: Atrazine catabolism pathway carried out by Rhodococcus sp. TE1. https://metacyc.org/pathway?orgid=META&id=PWY-5727&detail-level=4.

When these carriers are reduced, they gain electrons (and often hydrogen ions), becoming NADH from NAD+ and $FADH_2$ from FAD. The reduced carriers release electrons into mitochondrial membranes to obtain energy (ATP) through oxidative phosphorylation [50], [51]. *Actinomycetota* species use the same metabolic pathway to degrade 1,3,5,-triazine, it was studied in *Nocardioides sp. C190* [46], [52].

Several factors can influence the mineralization of atrazine by bacteria, enhancing their ability to break down this herbicide:

(1) **Soil Type**: Calcareous soils (rich in calcium carbonate) have been found to enhance atrazine degradation more effectively than clay loam soils [54].
(2) **Optimal Conditions**: The pH, temperature, and atrazine concentration significantly affect the degradation efficiency. Optimal conditions include a pH of around 3–7, temperatures around 25–35 °C, and moderate atrazine concentrations [55].
(3) **Presence of Metals**: The addition of certain metals, such as cobalt, magnesium, zinc, and iron, can enhance the degradation efficiency of bacteria because usually they are cofactors for enzyme function [55].
(4) **Carbon Sources**: The presence of simple carbon sources like glucose can influence the mineralization process. However, atrazine alone as a carbon and nitrogen source can also be effective [54].
(5) **Enrichment Cultures**: Using mixed enrichment cultures of microorganisms can accelerate atrazine

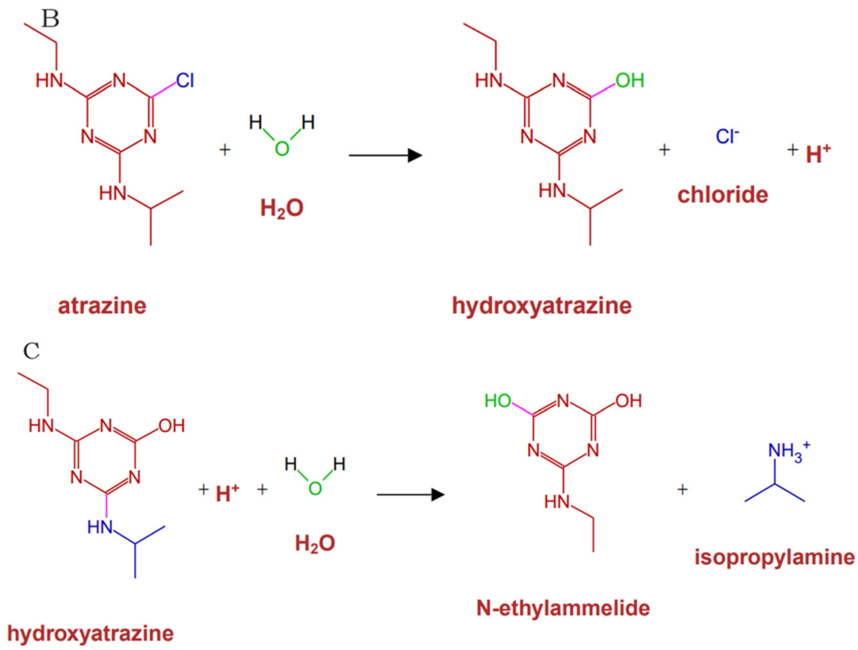


Figure 13: Reactions of atrazine to hydroxyatrazine and hydroxyatrazine to deethyl-atrazine, by Rhodococcus sp. TE1. https://metacyc.org/pathway?orgid=META&id=PWY-5727&detail-level=4.

mineralization. These cultures can transform atrazine into metabolites like hydroxyatrazine and eventually into carbon dioxide [55].

(6) **Genetic Adaptation**: Over time, bacteria can evolve to use atrazine as a growth substrate, developing new catabolic pathways for its degradation [56].

These factors contribute to the efficiency of atrazine mineralization by bacteria, making it a viable method for bioremediation of atrazine-contaminated environments.

6 Fungi's metabolic pathway

Biodegradation of atrazine by some fungi species had been reported too [29], [57]. Although fungi do not produce enzymes used by bacteria, their job in nature is to biodegrade lignin. Lignin is not a specific molecule, it is a very complex polymer formed mainly by six molecules bonded in random sort: sodium lignin sulfonate, dehydrodiconiferyl alcohol, veratrilc alcohol, lignin calcium sulfonate, lignin dealkaline, lignin organosolv, and lignin 94,113-57-2, see Table 2. Lignin recovers the truck and branches of plants.

Comparing the molecular structure of atrazine and lignin both are aromatic heterocycles, with nitrogen atoms inside the rings and electronegative atoms beside the rings, Chlorine in atrazine rings, and Oxygen in lignin rings. It is important to remember that the most electronegative element is fluor, with an electronegative value equal to 4.0; oxygen's electronegative value is 3.44, and chlorine's electronegative value is 3.16.

Fungi species are more efficient in the breakdown of lignin than bacteria (in bacteria delignification is slower and limited). Fungal is considered a good option for the remediation of herbicides because they produce no specific enzymes, able to break down more complex molecules than herbicides. As fungi's enzymes are not very specific, they can use atrazine as a substrate, and biodegradable it. Additionally, their mycelia can penetrate a great diversity of soils, they can grow on small surfaces adapting to fluctuating conditions like temperature, pH, and xenobiotics, furthermore, they are genetically stable [53].

Enzymes involved in lignin degradation can generally be divided into two main groups: lignin-modifying enzymes (LME) and lignin-degrading auxiliary (LDA) enzymes. LDA enzymes are unable to degrade lignin on their own, but they are necessary to complete the degradation process [54]. Enzymes involved in lignin degradation (LDA) are classified as phenol oxidase (laccases) and heme-containing peroxidase enzymes (POD) as: lignin (LiP), manganese (MnP), and versatile (VP) peroxidases [55]. Figure 14 shows the molecular structure of lignin peroxidase of *Phanerodontia chrysosporium* [58].

Lignin degradation requires hydrogen peroxide besides peroxidases (LiP, MnP, VP), to generate hydroxyl radicals. Hydrogen peroxide is a byproduct of LiP, MnP, and laccases. Hydroxyl radicals started the attack of lignocellulose. This group includes glyoxal oxidase, ary alcohol oxidases, pyranose 2-oxidase, cellobiose dehydrogenase, and glucose

Table 2: Monomers forming lignin. https://pubchem.ncbi.nlm.nih.gov/#query=lignin.

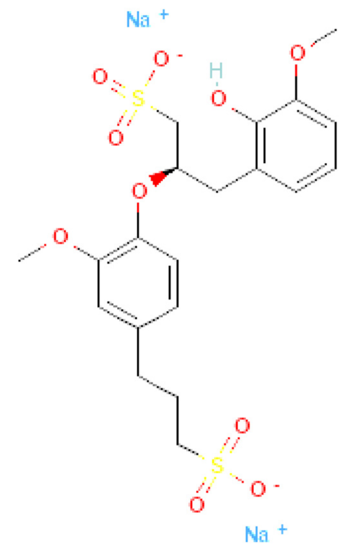
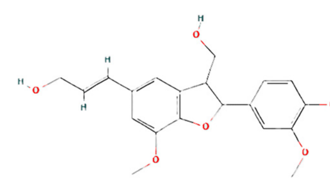
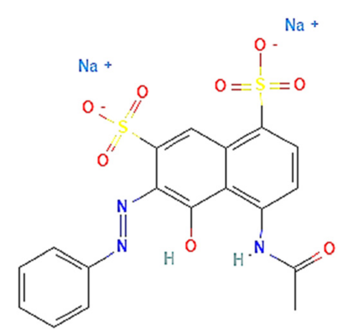
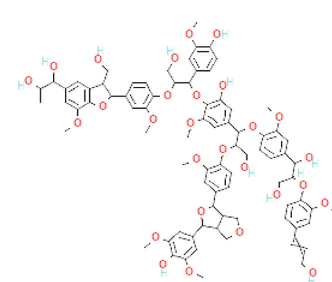
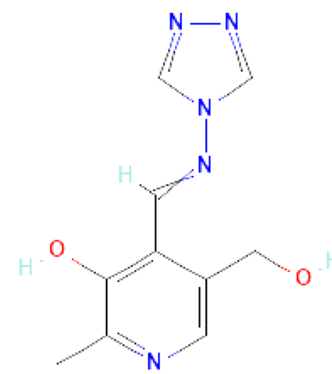
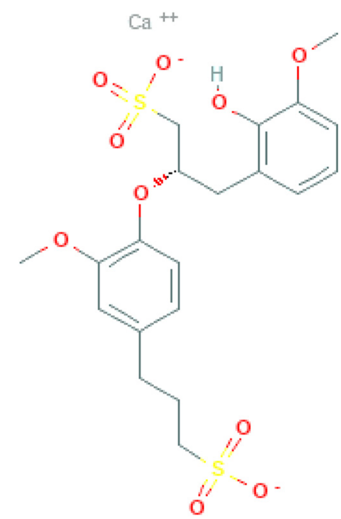
Name	Chemical formula	Molecu-lar weight	Chemical structure
Lignosul-fonic acid	$C_{20}H_{24}Na_2O_{10}S_2$	534.5	
Dehydro-diconiferyl alcohol	$C_{20}H_{22}O_6$	358.4	
Lignin 94,113-57-2	$C_{18}H_{13}N_3Na_2O_8S_2$	509.4	
Lignin, organosolv	$C_{81}H_{92}O_{28}$	1,513.6	

Table 2: (continued)

Name	Chemical formula	Molecu-lar weight	Chemical structure
Lignin dealkaline	$C_{10}H_{11}N_5O_2$	233.232	
Lignin calcium sulfonate	$C_{20}H_{24}CaO_{10}S_2$	528.6	

oxidase. These enzymes (LiP, MnP, VP) are frequently found in white-rot fungi secretomes [56]. On the other hand, laccases use molecular oxygen as electron acceptors while peroxidases use hydrogen peroxide as a co-substrate.

In nature, only *Basidiomycota* belonging to the aerobic white-rot group, this specie can degrade lignin completely. The enzymatic reaction that cleaves the aromatic ring requires oxygen or its partially reduced species. Anaerobic fungi lack the enzymatic machinery to catabolize/mineralize lignin [59].

Some fungi strains had been studied to biodegrade atrazine in liquid media, such as *Pluteus cubensis, Gloeophyllum striatum, and Agaricales*, they removed 30, 37 and 38 %, respectively, after 20 days, with an initial atrazine concentration of 25 mg L^{-1}. Those results were obtained in a culture media with Nitrogen deficiency (2.5 mM nitrogen concentration) [29].

Atrazine presence in culture media, induced the synthesis and secretion of extracellular laccases by *Pycnoporus sanguineus, Datronia caperata,* and *Polyporus tenuiculus*. Laccase levels produced were 13.3-fold superior in contaminated than in the control culture medium [29].

It had been reported that atrazine was used as a nitrogen source by a fungal strain purified from a cornfield soil that had been previously treated with herbicide. Approximately 34 % of atrazine was degraded in 20 days in a culture medium contaminated without a sterilizer. When the medium was sterile atrazine degradation was about 50 % in 4 days [2].

The bioremedial potential of white-rot fungi has been demonstrated with species such as *P. chrysosporium, Trametes versicolor, Bjerkandera adjusta, and Pleurotus sp.*, all of which produce ligninolytic enzymes, mainly laccases, and peroxidases. The enzymatic cleavage of the aromatic ring

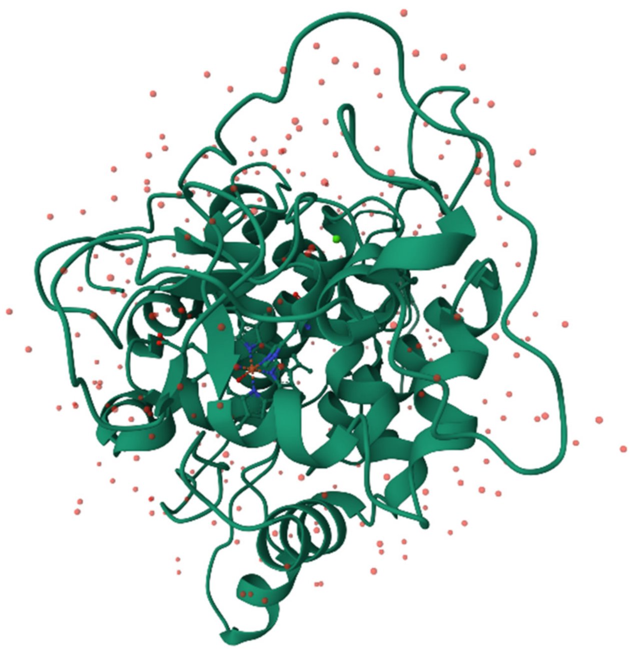


Figure 14: Lignin peroxidase of *P. chrysosporium* https://www.rcsb.org/structure/1b85.

requires oxygen, or some reactive oxygen species (OH^-, O_2^-, H_2O_2, $^1O_2$). Therefore, anaerobic microorganisms cannot degrade lignin.

Ligninolytic enzymes have been proven to degrade pesticides, dyes, phenolic compounds, colors, cresols, and petroleum hydrocarbons. In some cases, significant degradation was achieved by bio-stimulation with lignocellulosic substrate [60]–[62]. Fungus *Pleurotus ostreatus* was able to degrade 82 % of atrazine, 71 % of DEA, and 56 % of DIA after 22 days, with the hydrolases and peroxidase enzymes participation [3].

6.1 Lignin peroxidases

The lignin biodegradation peroxidase system consists of peroxidase enzymes, H_2O_2, veratryl alcohol, oxalate, and manganese-producing enzymes. All these enzymes are glycosylated heme proteins and are able to oxidate a variety of substrates with the reduction of H_2O_2. Lignin peroxidases and Manganese peroxidase have a higher redox potential than other peroxidases, this is why they can oxidize chemicals such as polycyclic aromatic hydrocarbon, phenol and its derivatives, cyanide, TNT, and others [63].

Peroxidases are oxidizing agents to initiate degradation reactions, commonly they begin with hydrogen peroxide to oxidize organic and inorganic substrates. Peroxidases are predominantly heme proteins containing protoporphyrin IX or iron III as a prosthetic group, they have molecular weights between 30 and 150 kDa; remember that molecular weights of enzymes are usually between 10 and 2,000 kDa, then they are little enzymes. LiP and MnP belong to the family of oxidoreductases [64].

Lignin peroxidase was identified in 1983, from *P. chrysosporium*, it is an extracellular enzyme, known as a diarylpropane-oxygenase or ligninase. There are different types of lignin peroxidases, such as LG2, LG3, H2, LG5, LG6, HB, A, and B; they are isomers. They have a molecular weight between 38 and 43 kDa. Lignin peroxidase (LG2) of *Phanerondontia chrysosporium* has three major alfa helices, eight minor helices, and three pairs of betas antiparallelly sheets [65]. Figure 15 shows the three-dimensional structure of LG2 of *P. chrysosporium*, Figure 16 is the 3-D representation of the heme group (active site) with Fe, and Figure 17 is the 2-D Molecular structure diagram of the heme group [66].

Peroxidases are not specific enzymes that can act over a wide variety of compounds, like phenols, aromatic amines, and polycyclic aromatic hydrocarbons. The main reaction catalyzed by peroxidases involves several steps and the formation of two oxidized intermediates. The first reaction is the interaction between hydrogen peroxide and the ferric (Fe III) atom of the heme group, producing an intermediate transient ferric hydroperoxide.

The optimum condition of lignin peroxidase for the removal of phenolic compounds is above pH = 4, it can be deactivated at lower pH levels [68]. Lignin peroxidase has a high redox potential (1.2 V at pH = 3) which makes it capable of oxidizing substrates that cannot be oxidized with other peroxidases [53]. LiP can oxidize the non-phenolic part of lignin. The catalytic cycle of LiP resembles the catalytic mechanism common to all peroxidases. In each cycle, the enzyme is oxidized by H_2O_2, and abstracts a single electron from lignin, generating a radical (compound I) intermediate that exists as a ferry oxo porphyrin radical cation [Fe (IV) = O^+]. Next, the enzyme is subjected to two single-electron reduction steps by the electron donor substrate, such as veratryl alcohol, leading to a transient formation of compound II [Fe (IV) = O] and a VA radical cation (VA^+). Compound II further oxidizes the second VA molecule, simultaneously returning to its native stage to initiate a new catalytic cycle of LiP. Like Mn (III), plays the role of being a small molecular weight redox transfer mediation between the enzyme and its polymeric substrate [59]. On the other hand, the lignin radical formed leads to the cleavage of various chemical bounds, including (C–O–C), and (C–C). Then lignin breaks down into two smaller molecules, which can be degraded later by other enzymes. Lignin peroxidase is effective in oxidizing non-phenolic lignin compounds, which are more resistant to degradation than phenolic

A

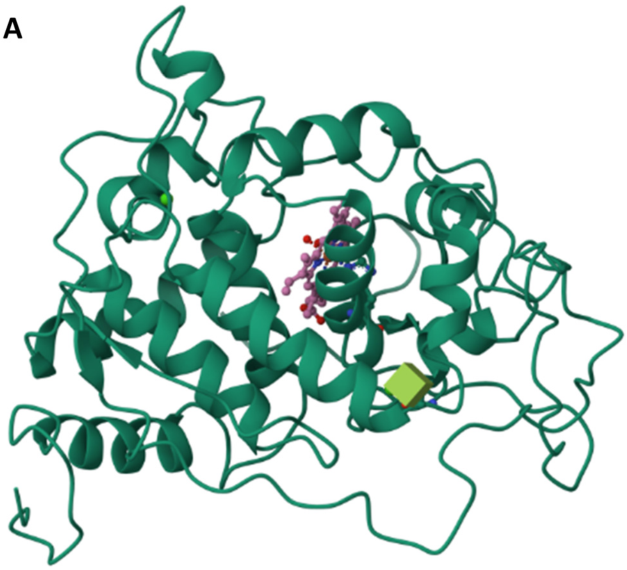


Figure 15: Lignin peroxidase of *P. chrysosporium* (LG3). Amino acids sequence with 371 units.

B

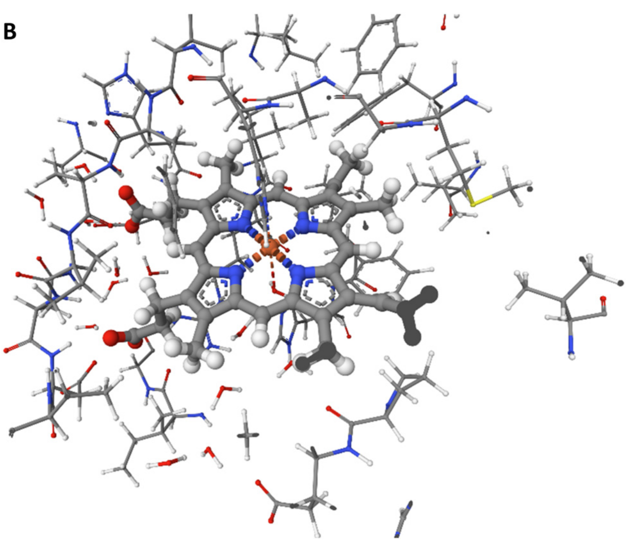


Figure 16: Lignin peroxidase of *P. chrysosporium* (LG3). 3-D representation of heme group (active site) with Fe.

compounds [67], [69]. Figure 18 shows the bioreaction mechanism to break down bounds among two rings, Figure 19 presents what occurs to lignin peroxidase enzyme with H_2O_2.

As can be seen, peroxidases cannot break down the rings of the lignin, they break the carbon bonds between monomers. Then peroxidases are not able to biodegrade atrazine. Anaerobic fungi lack the enzymatic machinery to catabolize/mineralize lignin [57].

For more than 30 years it was thought that only fungal produce LiP, however recently, it was found that some bacteria produced these enzymes too, like *Actinomycetes*, *α-Proteobaceria*, and *γ-Proteobacteria* [59], however, they cannot mineralize lignin. Fungi LiP is more potent than bacteria's LiP enzymes [70]–[72].

Recently research with complicated simulations has found that the heme group of lignin peroxidase of *P. chrysosporium* is inside a channel, and atrazine can't enter inside that channel by steric impediments. Then atrazine reacts with other active sites on the surface of the enzyme [73].

6.2 Laccases

Laccases are multicopper enzymes excreted by fungi species with the ability to form and destroy bonds as a function of the substrate and reaction conditions, they can catalyze polymerization (in plants) or depolymerization (in fungi) process. Their physicochemical properties such as activity, stability, molecular size, and isoelectric point are functions of their source [74]. Their amino acid sequence could be between 220 and 800, and they have molecular weights between 50 and 140 kDa and have alfa helices and beta sheets; their cofactor is copper [75]. The Laccase 3-D structure of *Phlebia radiata* is shown in Figure 20, [59].

They have a low redox potential, allowing direct oxidation of phenolic lignin units. An example is laccase from *Basidiomycete PM1,* it is a monomeric glycoprotein containing 6.5 % carbohydrates, it is stable in a pH range from 3 to 9, with an optimum pH level of 4.5 and temperature of 80 °C. It has a molecular weight between 55.5 and 64 kDa [35]. Laccases have four copper atoms in special oxidation states: one type I, one type II, and two type III, all forming their catalytic site, see Figure 21. Fungi's laccases oxidize a wide range of substrates, they substitute phenols and aromatic amines, which are transformed into free radicals. In *Pycnoporus cinnabarinus* there were found five types of laccases [76].

Laccase's reaction involves one electron (e^-), and the sequential oxidation of four molecules of reducing substrates, with two double electron reduction of oxygen atoms into their respective H_2O molecules. This process is accompanied by a catalytic exchange of 4 H^+ equivalents. However, its mechanisms and kinetic performance remain unclear [77].

Type I Copper is involved in electron transfer; type II Copper is involved in the reduction of oxygen to water, and type III Copper has two copper atoms that work together with type II, to reduce oxygen. The four copper atoms are located in the active site of laccases, they are responsible for catalyzing the transfer of electrons from substrate to molecular oxygen, to obtain water [78].

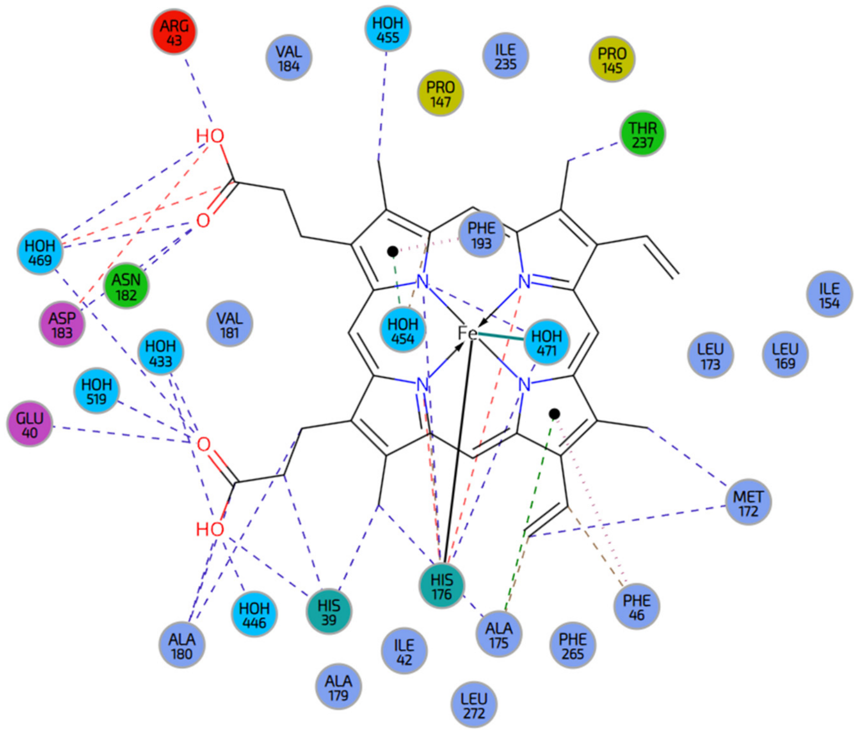


Figure 17: Lignin peroxidase of *P. chrysosporium* (LG3). 2-D molecular structure diagram of heme group [67]. Made with AlphaFold free simulation software.

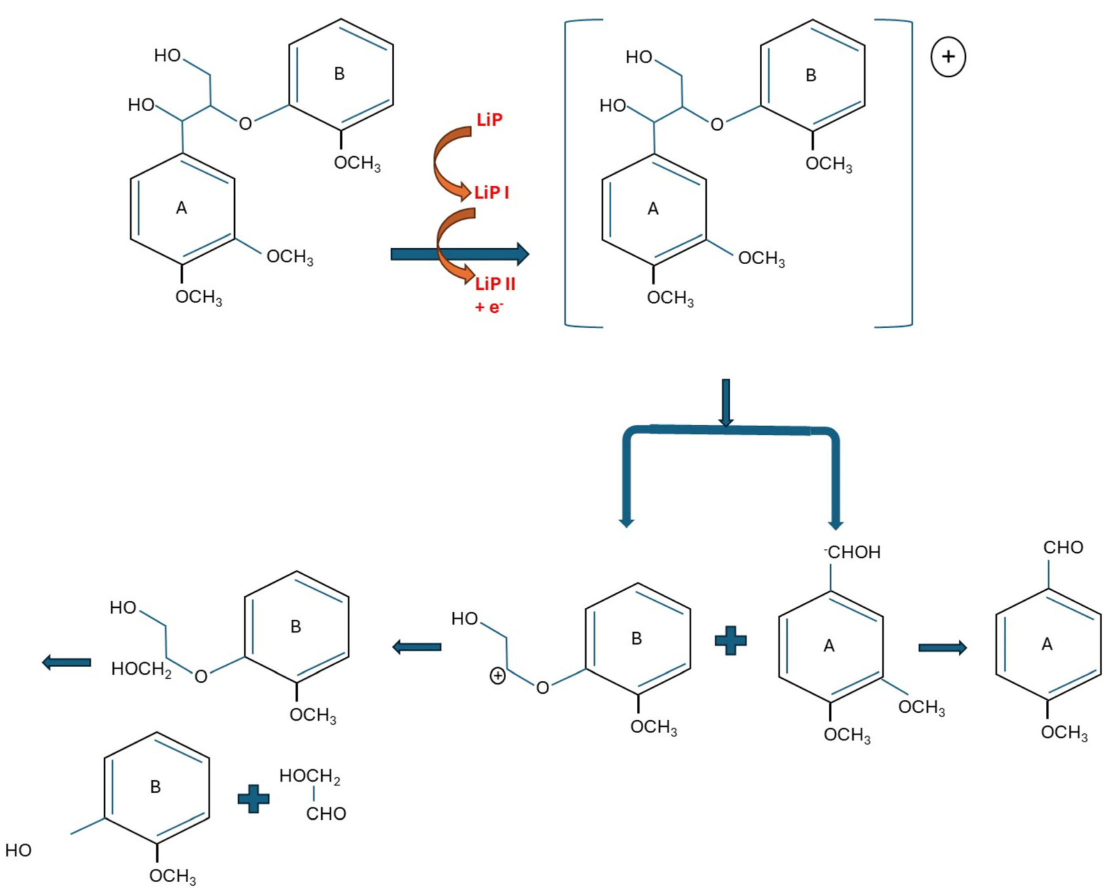


Figure 18: Mechanism reaction of lignin peroxidase [59].

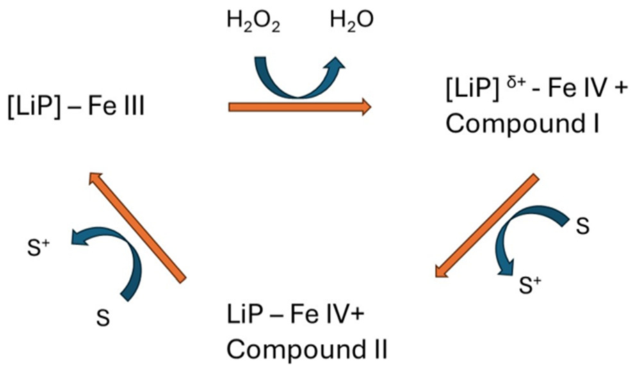


Figure 19: General schematic LiP cycle with H_2O_2 as oxidizing agent [67].

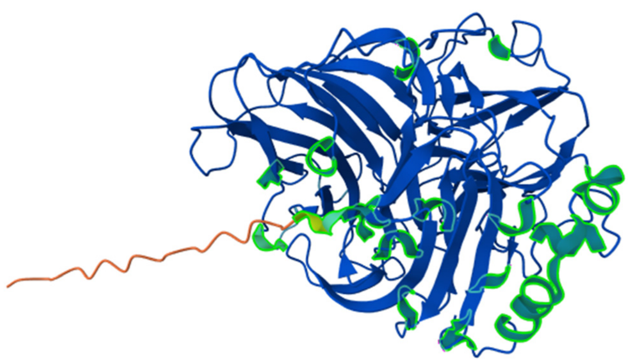


Figure 20: Laccase 3-D structure of *P. radiata*.

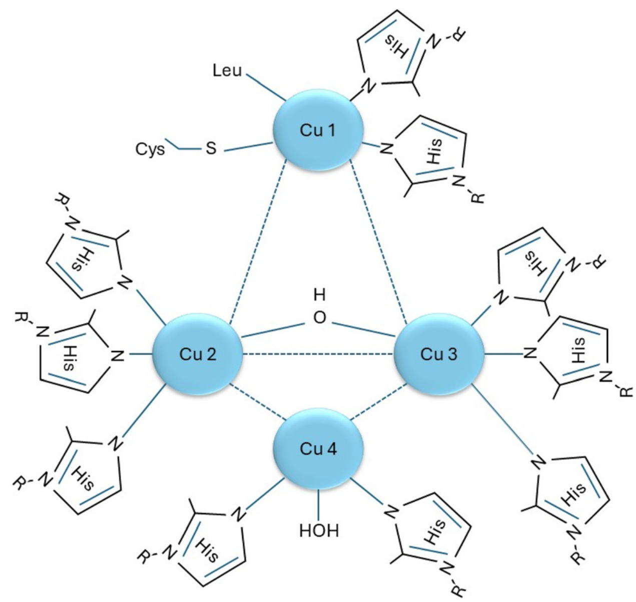


Figure 21: Laccase's general molecular structure. Cu 1 is type I, Cu 2 and Cu3 are type II, and Cu 4 is type III [79].

Laccases require oxygen to act and catalyze the direct and indirect oxidation reactions. The direct one oxidizes the substrate to its corresponding radical, by the interaction of copper cluster. If the ionization potential of the substrate is greater than the potential of copper type I, then it is necessary to have a mediator: first enzyme oxides the mediator, and then the mediator oxidizes the substrate, this is the indirect oxidation, see Figure 22.

Laccases oxidize a wide range of substrates, typically substituted phenols and aromatic amines, which are transformed into free radicals. Free radicals commonly start domino reactions leading to chemical transformations such as lignin synthesis and degradation [77].

Laccases catalyze, in general, catalyze the reaction of hydroquinone and oxygen to produce benzosemiquinone and water, see Figure 23.

Laccase from *Hericium coralloides* is stimulated by Mg^{2+} and Al^{3+} and inhibited by Fe^{2+} and Hg^{2+}. It exhibits oxidizing activity towards a broad range of substrates, in descending order, some of them are N, N-dimethyl-1,4-phenylenediamine, catechol, 2-methylcatechol, and pyrogallol, its optimum temperature is 40 °C and pH level is 2.2 [39].

The effect of adding ferulic acid and ethanol to culture media was tested to degrade atrazine. It was proved that adding 35 g/L of ethanol to culture media favored a continuous and high expression of the laccase gene, producing the isoenzyme laccase LAC I. The average yield of laccase with ethanol concentration was 1–1.5 g/L, showing much more production than culture media with ferulic acid [79].

LiP and laccases react with aromatic structure directly, but MnP forms Mn^{3+} ions from Mn^{2+} ions, as redox mediators. It is supposed that, in white-rot fungi, the primary attack on lignin is made by the MnP system [80]. As far as it is known, MnP is the only enzyme that mineralizes lignin partially outside the fungal hyphae. The intensity of the MnP system is enhanced by co-oxidants such as undersaturated fatty acids, because they are oxidized by Mn^{3+}, in the presence of oxygen, forming high reactive species like peroxyl and alkoxy radicals of fatty acids [81].

Henn et al., [29], measured the growth inhibition of different fungal strains in a PDA medium containing 6.25 and 10.0 mg L^{-1} of atrazine initial concentration. They found percentage inhibitions between 42 and 75 %. Furthermore, they found, that after 20 days, biomass production also decreased for most strains when atrazine concentration was 25 mg L^{-1}. Specifically, *P. sanguineus* growth inhibition was 68 and 69 %. Among 13 isolated species evaluated, 11 degraded atrazine in levels between 2.9 and 38.7 %. They quantified biomass concentration and laccase activity at atrazine initial concentration among 0 and 50 mg L^{-1}, using *P. tenuiculus*. They observed that there was growth inhibition increasing atrazine initial concentration, and that laccase activity increased with atrazine initial concentration [29].

Figure 22: Laccase's general reaction [68].

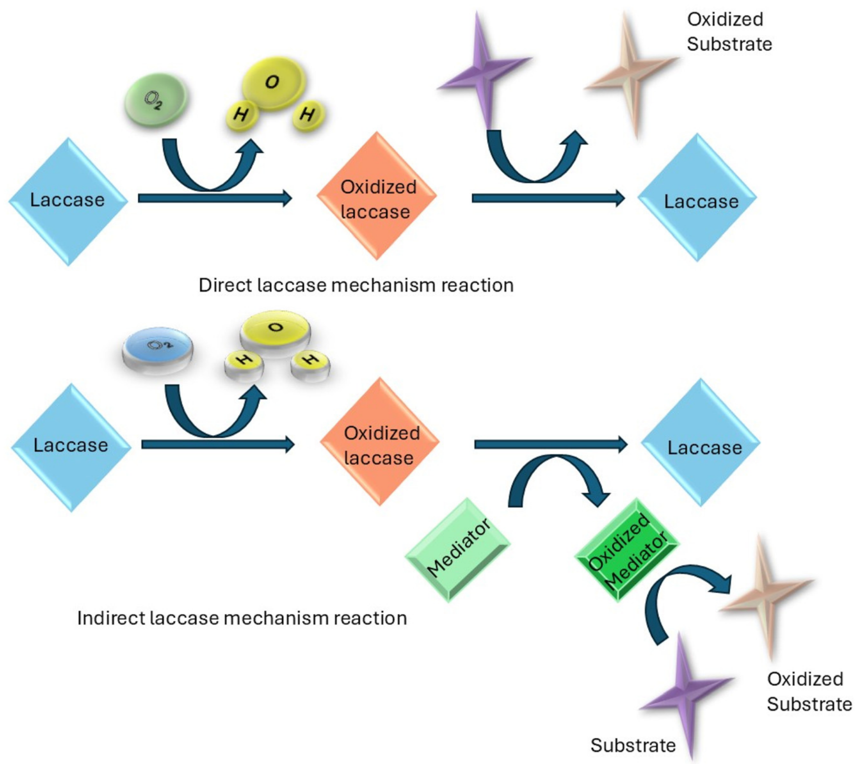


Figure 23: Reaction types of laccases, direct and indirect [79].

The presence of ethanol in culture media, in the concentration of 35 g L^{-1}, promotes laccase production until 1.0–1.15 g L^{-1} [79]. Ethanol in culture media upregulates the production of lignin peroxidases and laccases enzymes as part of a metabolic response because it creates a mild stress condition for the fungi, which can trigger the production of secondary metabolites, also ethanol can be used as an energy and carbon source by the fungi.

Manganese peroxidase production by white-rot fungi *Lentinus edodes* under high nitrogen concentration, was negligible, while it reached a maximum of 300 U mL^{-1} under low nitrogen conditions, after 12 weeks. Furthermore, manganese peroxidase is affected by the Mn concentration in culture media, with the highest enzyme levels recorded at 1.1 ppm [74].

Aspergillus niger, a filamentous fungus was used to biodegrade atrazine with *O. ficus indica* as co-substrate, reaching biodegradation in order of 70–75 % in six days, with an initial atrazine concentration of 1 g L^{-1} [58].

Several factors can enhance the mineralization of atrazine by fungi such as:

(1) **Oxygen.** The lack of oxygen prevents the oxidation reactions that degrade the atrazine from taking place.
(2) **Presence of Mn, Fe, Cu and Ca.** The presence of manganese and iron are very important factors because they are essential for the production of laccases and lignin peroxidases respectively. The presence of copper is important because it is a cofactor for laccases. Calcium is required to give structural stability to lignin peroxidases.
(3) **Soil Type**: Soils rich in organic matter and microbial biomass tend to support better atrazine degradation. For example, soils with higher organic carbon content and lower atrazine application history show increased mineralization rates [82]. The optimal C/N relation is an important factor that is between 20 and 30, because if there is more nitrogen the fungi metabolic pathways are directed towards biomass production.

(4) **Optimal Conditions**: The pH, temperature, and moisture content of the soil can significantly affect fungal activity and atrazine degradation. Optimal conditions are pH slightly acidic pH because the isoelectric point of LiP and MnP is between 4.7 and 3.1 and these pH conditions help enzyme to conserve their dimensional structure. Moderate temperatures (around 25–35 °C), and adequate moisture levels. However, it is important to use the optimal pH and temperature for the specific species.
(5) **Co-substrates**: The presence of additional carbon sources can enhance fungal growth and atrazine degradation. Simple carbon sources like glucose or ethanol can be beneficial, also lignin high content sources such as straw, *O. ficus indica* residues, or sawdust have been reported.
(6) **Presence of inhibitory substance**: High atrazine concentrations can inhibit the growth and function of some fungi species; it is necessary to find the toxicity limits for each one. Other heavy metals like cadmium, lead, and mercury can be toxic to fungi, inhibiting their growth and enzyme production. High concentrations of phenolic and organic solvents like benzene and toluene can disrupt fungal cell membranes and then metabolic processes.
(7) **Enrichment Cultures**: Using mixed cultures of fungi can improve atrazine degradation rates. These cultures can transform the atrazine into less harmful metabolites and eventually into carbon dioxide.
(8) **Genetic Adaptation**: Over time, fungi can adapt genetically to use atrazine as a carbon and nitrogen source, developing new catabolic pathways for its degradation.

These factors contribute to the efficiency of atrazine mineralization by fungi, making it a viable method for bioremediation of atrazine-contaminated environments.

Figure 24 shows a proposal of a metabolic pathway that could be followed by atrazine when it is mineralized by fungi's enzymatic system. Considering that the enzymatic system produces a lot of radicals OH^-, and that chlorine in atrazine molecules is less electronegative than oxygen in radical OH^-, it is probable that a chlorine atom could be substituted by an OH in atrazine, as the first step, forming hydroxy-atrazine plus hydrochloric acid. In a second step, isopropylammelide could be formed by breaking the bond between the aromatic ring and ethylamine and substituting ethylamine with another OH group. We think that it is easier to break the bond between ethylamine and the ring than between propylamine and the ring; because the smaller and less branched groups are easier to oxidize since their smaller size allows easier access to the reaction center.

The third step could be the breaking of the bond between ethylamine and ring, and the substitution of ethylamine by an OH group, forming cyanuric acid plus propylamine. The next step proposed is breaking the ring to 1-carboxy-biuret, the presence of the three OH groups increase the electron density of the ring, making it more susceptible to oxidation reactions and consequently facilitating the cleavage of the aromatic ring. Then could begin three consecutive oxidation reactions including the forming of three carbon dioxide, and three ammonia molecules. This pathway is already like bacteria because all are oxidation reactions. However, there is no experimental evidence that proves this pathway in fungi.

Fungal cell walls are dynamic structures, like other organelles, whose composition is regulated by the environment. They are malleable and mechanically robust [83], give protection and structure to the cells, and represent a permeability barrier, that allows the entrance of some substances inside the cell [84]. Atrazine and all the metabolites shown in Figure 24 can cross the hyphae's wall cells of the

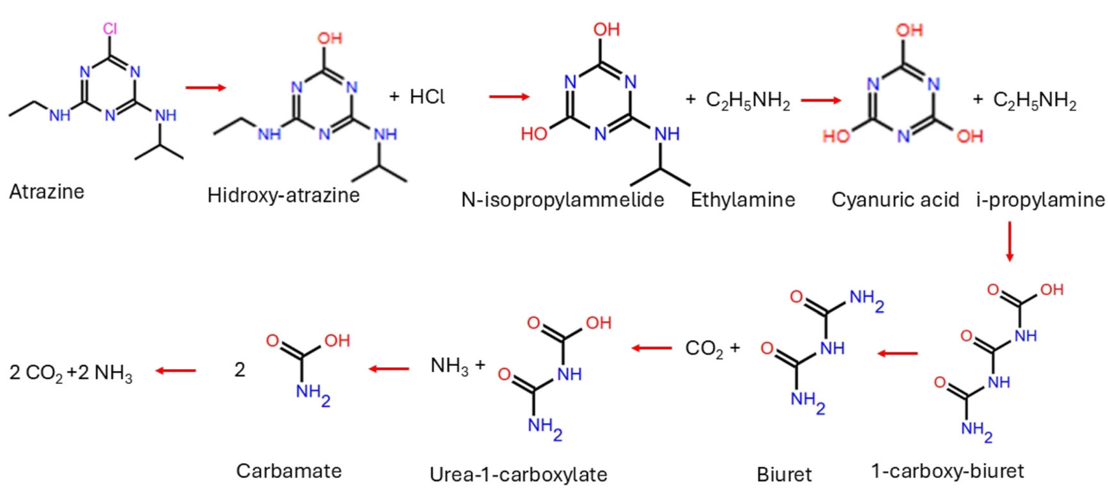


Figure 24: Proposal of metabolic pathway atrazine biodegradation by fungi's laccases and peroxidases.

fungus. The mechanism includes the formation of liposomes or vesicles outside the wall that involve the atrazine and go inside the cell by the viscoelastic properties of the wall cells.

On the other hand, LiP, MnP, VP, and laccases are synthesized inside the cell in the ribosomes of the endoplasmic reticulum. Then, they are transported through the Golgi apparatus, where they are involved in secretion vesicles. Those vesicles fuse with membrane cells and are excreted to the medium. Then it is not very clear if atrazine is oxidized outside cells by the exoenzymes, and later some of the metabolites cross the wall cell and are degraded by hydrolases and oxidoreductases enzymes inside the cell, both, atrazine and cyanuric acid require facilitated transport to cross the wall cell, this transport type requires energy expenditure. The energy expenditure required to cross the cell membrane depends on temperature, inside and outside cell concentrations, composition of wall cell, and size and polarity of the substrate. As temperature, inside and outside concentrations of atrazine and cyanuric acid, and wall cell composition are the same, the parameters that can be different are size and polarity between atrazine and cyanuric acid. Atrazine's size and polarity are bigger than cyanuric acid, then it is probable that cyanuric acid transportation through wall cell requires less energy than atrazine's. Then, it is likely that atrazine is degraded to cyanuric acid outside the cell and this enters the cell, where the rest of the reactions take place. Then nitrogen and carbon contained in the atrazine can be used by the cell in other metabolic pathways.

Besides, since the active site of lignin peroxidases is in the center of the enzyme, and it generates compound I (see Figure 24), it is probably that compound I travels inside the channel mentioned above toward the atrazine molecule, because there is new evidence that atrazine cannot go inside the channel by steric hindrances.

7 Discussion

Biological methods are characterized by their cost-effectiveness good relationship, to be applicable on a broad scale, simple application procedure, minimal environmental impact, and the absence of toxic byproducts (if the microorganism can mineralize atrazine). They can be used directly on soil and contaminated water, and in bioreactors. The efficiency of these methods is determined by a lot of factors when they are used directly, like temperature, salinity, pH, nutrients, co-substrates, and toxic substance content, among them initial atrazine concentration. However, they are slower than physicochemical methods.

In comparison, physicochemical methods require large investments in process plants, transportation of soil or water to treatment plants, and catalysts, and consume much more energy than biological processes.

The degradation rate is compared in Table 3 for biological bacterial and fungi methods and some physicochemical systems. The degradation rate was calculated with equation (18).

Table 3: Comparative degradation rate between bacterial, fungi, and physicochemical processes.

Method Biological	Removal (%)	Time (days)	Initial conc. (mg/L)	Final conc. (mg/L)	Degrading rate (mg/Ld)	Reference
Pseudaminobacter strain C147[a]	95.24	0.8	9.05856	0.43136	10.90	[85]
Nocardioides strain C190[a]	78.57	0.8	9.05856	1.94112	8.99	[85]
Pseudomonas strain ADP[a]	88.09	0.8	9.05856	1.0784	10.08	[85]
Agrobacterium radiobater J14a[a]	94	3	5.00E-02	4.70E-02	0.001	[26]
Pseudomonas[a]	91.8	44	50	4.10E-01	0.10	[86]
Citrococcus sp. Strain TT3[a]	100	2.75	50	0.00	18.18	[87]
Pluteus cubensis[b]	30	20	25	17.5	0.38	[29]
Gloelophyllum striatum[b]	37	20	25	15.75	0.46	[29]
Agaricales[b]	38	20	25	15.5	0.48	[29]
Polyporus[b]	82	22	3	0.54	0.11	[29]
A. niger[b]	75	6	300	75	37.50	[57]
Gloelophyllum striatum MCA7[b]	*37.3*	*20*	*25*	*15.67*	*0.47*	[29]
Physicochemical						
Photocatalylic (ACF/CoFe2O4)	86.7	0.625	15	1.995	20.81	[88]
Electrochemical	90.1	0.073	10	0.99	123.57	[89]
Fenton with AFG@MIL-101(Fe)	81	0.073	30	5.7	333.26	[90]
Fenton with nZV1/H_2O_2/UVA	80	0.0833	10	2	96.00	[91]
Adsorption onto polyethylene	0.1	1	15	14.985	0.02	[92]
Adsorption onto graphene	100	0.375	100	0	266.67	[93]
Adsorption onto biochar	52	10	6	2.88	0.31	[94]

[a]Bacteria species, [b]Fungi species.

$$8 \cdot r_A = \frac{C_{A0} - C_A}{t} \tag{18}$$

where C_{A0} is the atrazine's initial concentration, C_A is the atrazine's final concentration and t is the duration of each experiment reported, results are shown in Table 3. As can be seen, the bacteria species' greatest degradation rate reported is 19 mg (L d)$^{-1}$, Fungi species maximum degradation rate is three times higher than bacteria, and the physicochemical method Fenton can reach up to 333 mg/L d, which means ten times higher than fungi's species.

8 Conclusions

This review summarizes the recent studies about metabolic pathways and enzymes to degrade atrazine to carbon dioxide, ammonia, water, and biomass carried out by some bacteria and fungi species. Both bacteria and fungi-specific species have the potential to oxidize atrazine because they have the ability to use the enzymatic systems that intervene in the metabolic pathways responsible for degrading atrazine.

Chemical bioremediation methods could be used only if atrazine is in a liquid media, a previous step is required to extract atrazine from soil if atrazine is in the soil. Later, it is necessary to use special equipment to degrade atrazine and clean the water used to dissolve it. Special equipment could be a reactor, a photoreactor or an electrochemical cell. Moreover, almost all physicochemical methods require chemical compounds like catalysts, coatings for electrodes, or oxidizing agents like ozone.

Physical methods require adsorbent materials where atrazine is contained without suffering any chemical transformation, next step is necessary for desorbing atrazine, usually changing pressure, after we have a stream with atrazine that is necessary to degrade by chemical or biological processes.

Biological processes are made by some bacteria and fungi-specific species. Only some Gramm-positive bacteria have the enzymatic system necessary to degrade atrazine. White rot fungi species have the ability to degrade lignin naturally, if they have the enzymes to degrade lignin can degrade atrazine, for its similar and simpler chemical structure. All of these methods are aerobics.

It is important to consider that biodegradation of atrazine can produce more toxic end products than atrazine, then it is necessary to look for the microorganism that can mineralize atrazine to biomass, carbon dioxide, ammonia, and water. The Bacteria species reported that can mineralize atrazine are *Arthrobacter sp*., *Pseudomonas sp*., and *Bacillus sp*. Only white-rot fungi species such as *Basidiomycota, Pluteus, Gloelophylum, Pycnoporous*, and *Datronia* can mineralize atrazine.

As white-rot fungi are a good option for lignin biodegrading they can oxidize atrazine molecules too and break down its ring, because atrazine has a similar molecular structure to lignin, but much simpler. Furthermore, as peroxidases, that have a strong catalytic potential to break down C–O–C and Cα – Cβ bounds, and laccases that destroy double bonds, are not specific enzymes, they can biodegrade atrazine. When ligninolytic enzymes are used to degrade atrazine is essential to take into account that it is necessary to ensure that nitrogen concentration in soil is much lower than the required by the fungi, because obtaining nitrogen for leaving is the goal to degrade atrazine.

Although some microorganisms couldn't mineralize atrazine, if they can produce some intermediate compounds such as cyanuric acid *(Pichia kudriavzevii)* [95] they could be used with microbial consortia. Biological biodegradation processes can be made in soil or in liquid phases, and in bioreactors or in soils directly.

Physiochemical degradation processes require a higher investment than biological processes, however they are much faster than biological processes.

Acknowledgments: The authors acknowledge our institutions for the help they gave us in preparing this manuscript: especially to: the Process Analysis Area, the Biotechnology and Energy Departments, and the Chemistry Institute.
Research ethics: Not applicable.
Informed consent: Not applicable.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interest: All authors state no conflict of interest.
Research funding: None declared.
Data availability: Not applicable.

Nomenclature table

atzA	atrazine chlorohydrolase
atzB	hydroxyatrazine ethylaminohydrolase
atzC	N-isopropylammelide isopropylaminohydrolase
atzD	cyanuric acid amidohydrolase
atzF	allophanate hydrolase
C_A	atrazine's final concentration

C_{A0}	atrazine's initial concentration
d	day
DACT	diamino-cholorotriazine
DEA	De-ethyl-atrazine
DEDIA	De ethyl-de-isopropyl-atrazine
DEIA	De-isopropyl-atrazine
DIDEA	dialkylated atrazine
Gstd	Gibbs energy at standard conditions
Hh	biuret amidohydrolase
HIATZ	1-hydroxy-isopropyl-atrazine
HyA	hydroxy-atrazine
kDa	kilodalton
L	liter
LDA	lignin-degrading auxiliary
LiP	lignin peroxidase
LME	lignin-modifying enzymes
mg	milligrams
MnP	manganese peroxidase
POD	heme containing peroxidase
r_A	atrazine degradation rate
Symbol	definition
trzN	triazine hydrolase
VP	versatile peroxidase

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