**Applications of white rot fungi in bioremediation with nanoparticles and biosynthesis of metallic nanoparticles**

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**Abstract**

White rot fungi (WRF) are important environmental microorganisms that have been widely applied in many fields. To our knowledge, the application performance of WRF in bioremediation can be greatly improved by the combination with nanotechnology. And the preparation of metallic nanoparticles using WRF is an emerging biosynthesis approach. Understanding the interrelation of WRF and nanoparticles is important to further expand their applications. Thus, this mini-review summarizes the currently related reports mainly from the two different point of views. We highlight that nanoparticles as supports or synergistic agents can enhance the stability and bioremediation performance of WRF in wastewater treatment, and the biosynthesis process and conditions of several important metallic nanoparticles by WRF. Furthermore, the potential toxicity of nanoparticles on WRF and challenges encountered are also discussed. Herein, we deem that this mini-review will strengthen the basic knowledge and provide valuable insight for the applications of WRF and nanoparticles.

Keywords: White rot fungi; Bioremediation; Biological resource; Nanoparticles

**Introduction**

White rot fungi (WRF) are one of the most important microorganisms in natural environment. They are well known for their powerful enzyme system capable of degrading lignin and carbohydrates such as cellulose and hemicelluloses in wood, which is pivotal to the forest biogeochemical cycles (Hunt et al., 2013; Hervé et al., 2014). Meanwhile, WRF are versatile and robust microorganisms that have enormous potential for environmental remediation (Wesenberg et al., 2003; Asgher et al., 2008). Hitherto, they have been widely applied in organic pollutants wastewater treatment. It has been already demonstrated that the effective biodegradation capacity is ascribed to the non-special nature of produced extracellular enzyme complex containing lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), and laccase, which can degrade a variety of xenobiotics and recalcitrant pollutants with compound aromatic structures (Pointing, 2001; Wesenberg et al., 2003; Leeet al., 2014). Moreover, it has also reported the wide use of WRF in bioremediation of heavy metals or the composite-pollutants wastewater (Bayramoğlu et al., 2003; Chen et al., 2011; Chen et al., 2012; Huang et al*.*, 2015a). Therefore, the special capacities of WRF have aroused extensive research interest in the field of industrial/environmental microbiology. Nevertheless, the limitations for their practical applications in bioremediation still exist. The major problems are how to enhance their stability and resistance against environmental disturbances during the process of wastewater treatment (Chen et al., 2013). To overcome these challenges, the immobilization technology has been proposed as an effective method to improve the practical performance of WRF. Indeed, the immobilized microbial cell systems have attracted extensive concerns, mainly due to the distinct advantages over the freely suspended cells such as enhanced mechanical strength, ease of regeneration, and easier solid-liquid separation (Bayramoğluet al., 2003; Xu et al., 2012a). Excitingly, with the rapid development of nanoscience and nanotechnology, nanoparticles as support carriers of microbial cell systems have exhibited great potential in bioremediation (Xu et al., 2012a; Hou et al., 2014). In addition, nanoparticles also can act as synergist to improve the bioremediation capacity (Li and Zhang, 2016; Huang et al., 2017). However, to our knowledge, there is only scattered information that addressed the application of WRF with nanoparticles in environmental field. Thus, it is important to fully understand the increased bioremediation performance of WRF with the aid of nanoparticles.

In addition to the bioremediation in environmental field, WRF have also been considered as a promising biological resource for biosynthesis due to the successful preparation of metallic nanoparticles. For example, stable CdS nanoparticles could be synthesized when *Trametes versicolor* challenged with toxic cadmium ions through *in situ* reducing (Sanghi and Verma, 2009a), which highlighted the potential of WRF not only in bioremediation, but also in large-scale biosynthesis of metallic nanoparticles. Generally, the traditional physical and chemical methods are used for the synthesis of nanoparticles. However, their drawbacks such as low efficiency and the generation of hazardous wastes may costly and negatively impact the environment (Kalishwaralal et al., 2010; Zhang et al., 2011). Furthermore, many additional measures are needed to solve the potential problems such as stability of nanoparticles preparation and aggregation of particles (Sanghi and Verma 2009). Hence, it is necessary to develop high-yield, low-cost, and environment-friendly approaches for the systhesis of metallic nanoparticles. Thus, there is an increasing need for the research of biosysthesis. To date, a wide range of biological resources available in nature from simple prokaryotic bacterial cells to eukaryotic fungus and even live plants have been employed for synthesis of nanoparticles (He et al., 2007; Sanghi and Verma, 2009b). Among the biological systems, filamentous fungi are the most commonly used microorganisms resources due to their ease of handling and culturing, high tolerance towards metals, and wall-binding capacity for biosynthesis (Dhillonet al., 2012; Yadav et al., 2015). Given that WRF are ubiquitous in natural environment, more importantly, the biological approach for the synthesis of metallic nanoparticles without additional stabilizer and plays part roles in bioremediation (Sardar et al., 2014; Thakkar et al., 2009; Sanghi and Verma 2009), the application of WRF for biosynthesis will obtain increasingly attention in future work.

In order to enrich the knowledge of the application of WRF in the bioremediation and biosynthesis, the recent related research reports are summarized in this mini-review. Specially, the emphasis is focused on the link between WRF and nanoparticles from two different point of views, mainly including the wastewater treatment by WRF combined with various nanoparticles and the role and mechanism of WRF in biosynthesis of metallic nanoparticles. In addition, the effects of several certain nanoparticles on the growth of WRF need special attention, owing to the bioremediation of WRF will be affected in the presence of nanoparticles (Zuo et al., 2015; Huang et al., 2017). We, herein, deem that this mini-review will provide the practical guide for the application of WRF and enhance their link with nanoparticles.

**Bioremediation of white rot fungi combined with nanoparticles**

WRF have been widely applied in bioremediation for wastewater treatment. With the development of nanotechnology, the application of nanomaterials in environmental field is also increasing. The combination of biotechnology and nanotechnology is an emerging approach for bioremediation, which has exhibited great potential in environmetal remediation. As expected, the practical performance for pollutants removal using WRF biomass is increased significantly after combining with nanopaticles in environment (Chen et al., 2013; Xu et al., 2013; Hu et al., 2016). Therefore, in this section, the recent reports regarding the studies on the combination of WRF or their enzymes with nanoparticles in environment remediation are summarized.

Immobilization is a general term that describes many different forms of cell attachment or entrapment (Cassidy et al., 1996). Nowadays, the use of immobilized cells has been regarded as an effective alternative for environment application, because the immobilization technology can enhance the ability of microbes against the environmental perturbations by increasing the mechanical strength and providing the convenience for regeneration (Xu et al., 2012a). Magnetic nanoparticles have gained considerable attentions as immobilization carriers for WRF in wastewater treatment due to their high surface area and unique superparamagnetism that easy for separation (Xu et al., 2012b). Xu et al. ([2013](#OLE_LINK40)) prepared a biosorbent by the immobilization of *Phanerochaete chrysosporium* with iron oxide magnetic nanoparticles (MNPs) and Ca–alginate for Pb(II) removal. They found that the as-prepared novel adsorbent (MNPs–Ca–alginate immobilized *P. chrysosporium*) showed higher adsorption capacity (185.25 mg/g), which was about 5 times and 2.3 times that of pure MNPs and free *P. chrysosporium*. Shi et al. ([2014](#OLE_LINK41)) applied concanavalin A-activated Fe3O4 nanoparticles (GAMNs-Con A) as carrier to orient immobilization of laccase from *Echinodontium taxodii*. Consequently, this composite had higher enzyme loading and activity recovery, and showed higher removal rate of sulfonamide antibiotics. Huang et al. (2006) prepared copper tetra-aminophthalocyanine (CuTAPc)–Fe3O4 nanoparticle composite as the support of *Pycnoporus sanguineus* laccase, which could improve the thermal and storage stability of laccase remarkably. Meanwhile, they found that immobilized laccase still retained 80% of its initial activity after five consecutive operations. Wang et al. (2013) selected magnetic Fe3O4/SiO2 nanoparticles with particle size below 30 nm as support to immobilize laccase (IM-laccase) for the decolorization of phenolic azo dyes. As expected, there was no color change of Procion Red MX-5B and azophloxine in free laccase treatment, owing to the two dyes are not the substrates of laccase. However, the decolorization percentage of each dye was higher than 80% within 1 h when using IM-laccase. Additionally, Huang et al. (2015b) prepared a novel composite system containing Fe3O4 nanoparticles and *P. chrysosporium* together with its secretion oxalate, which could effectively degrade phenol via the coupled photocatalytic-biological process under solar light. The possible degradation mechanism was shown in Figure.1. On one hand, Fe3O4 nanoparticles could absorb oxalate secreted by *P. chrysosporium,* then they would react to form the hydroxyl radical (•OH) with higher redox potential under light condition, which could effectively enhance the phenol degradation; on the other hand, LiP and MnP could also catalyze the degaradation of phenol. This finding proposed a new combination approach for wastewater treatment. The great potential of magnetic nanoparticles for improving the stability and bioremediation performance of WRF, making the magnetic nanoparticles immobilized WRF increasingly popular in environmental field.

TiO2 nanoparticles are another potential ideal candidate for the immobilization due to their unique properties including high mechanical strength, low price, physical and chemical stability, low toxicity, as well as good biocompatibility (Hou et al., 2014). In 2013, [Chen et al](#OLE_LINK17). reported that the immobilized *P. chrysosporium* loaded with nitrogen-doped TiO2 nanoparticles (PTNs) could effectively treat the wastewater containing 2,4-Dichlorophenol (2,4-DCP) and cadmium, and the immobilization could not only enhance the resistance of *P. chrysosporium* to the toxics, but also shorten the treatment time. Commonly, the exposure to toxic pollutants will disrupt the structure of membrane phospholipids, induce the lipid peroxidation and the generation of reactive oxygen species (ROS) including superoxide anion (•O2-), hydrogen peroxide (H2O2), and hydroxyl radical (•OH), as well as the subsequent oxidative stress, which will cause the cell damage and death eventually (Chen et al., 2014a; De et al., 2013). Oxidative stress is a disorder caused by the excess ROS, which will cause the oxidative damage due to the imbalance between ROS generation and elimination. To prevent the oxidative damage, cells have developed the antioxidative defense system including the antioxidant enzymes such as catalase, superoxide dismutase. To further illuminate the resistance mechanisms of PTNs after exposure to toxic pollutants, their group investigated the change of physiological fluxes and antioxidative enzymes activities in 2015. In their work, significant changes in the physiological (H+, O2, and Cd2+ ) fluxes were observed at early cellular stress response to 2,4-DCP and Cd2+, and the resistance of PTNs to the toxic pollutants was ascribed to the efficient response to oxidative stress (Tan et al., 2015). Similarly, Hu et al. ([2016](#OLE_LINK37)) reported that the immobilized *P. chrysosporium* loaded with nitrogen-doped TiO2 nanoparticles was effective for the treatment of landfill leachate with a very low biodegradability ratio (BOD5/COD) of 0.09. In addition, heavy metals were also removed partly during the process of landfill leachate treatment. Hou et al. (2014) prepared a biocatalytic membrane via the immobilization of laccase on TiO2 blended polyethersulfone (PES) membranes, which took the advantages of both nanoparticles and membrane filtration system. Importantly, this as-prepared biocatalytic membrane showed better enzyme stability, higher tolerance to pH range and vigrous filtration conditions compared with packed bed and batch reactors. As is well known, TiO2 is one of the most widely used semiconductor catalysts that can photo-catalytically degrade organic compounds into harmless inorganic compounds (Daghrir et al., 2013). Since the phenol pollutants can be degraded under the action of Fe3O4 nanoparticles and *P. chrysosporium* via the coupled photocatalytic and biological process under light condition, which has been verified by Huang’s group (2015b), we deem that the further studies on the photocatalytic-biological process and mechanism of TiO2 immobilized WRF for organic pollutants degradation should be carried out.

Moreover, the elemental selenium nanoparticles immobilized in *P. chrysosporium* pellets displayed higher adsorption capacity for zinc removal due to more sorption sites on this adsorbent (Espinosaortiz et al., 2016). It has determined that the immobilization of WRF on nanoparticles is more efficient for pollutants removal. However, the presence of sole nanoparticle is prevalent in the environment, how they will impact the removal performance of WRF on the pollutants is remained to be explored. Recently, γ-Fe2O3 nanoparticles were found to enhance the bisphenol A (BPA) degradation with WRF, *Pleurotus ostreatus*, in the presence of H2O2. The phenomenon was attributed to their bioreduction to Fe3O4 by the extracellular Fe3+-reducing compounds excreted by *P. ostreatus*, which was more efficient for catalyzing the Fenton reaction to produce the •OH radicals. The increased number of active •OH radicals resulted in the accelerating BPA degradation. (Li and Zhang, 2016). The proposed facilitated mechanism was shown in Figure.2. Recently, our research group also determined that an appropriate concentration of AgNPs (0.1 mg/L to 1 mg/L) added into the solutions could increase the removal capacity of Cd2+ with *P. chrysosporium* (Zuo et al., 2015); similarly, a dosage of AgNPs range from 1 to 60 µM could enhance the degradation ability of *P. chrysosporium* to 2,4-DCP (Huang et al., 2017). Thus, aside from being support of WRF, the nanoparticles as synergistic agents that enhance the removal performance of WRF are also worth to explore in the future work.

Overall, the combination of WRF and nanoparticles can play an important role in wastewater treatment. In order to further enhance the bioremediation performance of WRF, the modified nanoparticles with special capacity (such as magnetic, photocatalysis, multi-adsorb sites, etc.) should be taken into account. Thus, considerable efforts should be devoted to the development of the combination of environmental microbiology and nanoscience.

**Biosynthesis of metallic nanoparticles by white rot fungi**

Due to the unique properties in chemistry, optics, electronic, and magnetic, nanoparticles have been attracting great interest in their synthesis and applications (Zhang et al., 2011; Liu, 2006). Biosynthesis is a high yield and eco-friendly approach for the preparation of nanoparticles. With the growing success and the nonpathogenic nature of WRF for producing nanoparticles, they have been seen as an interesting biological resource. We have tabulated these various WRF exploited for the synthesis of different metallic nanoparticles (Table 1). In this section, we have focused on the WRF as an important tool for the fabrication of several important metallic nanoparticles. In addition, methods, mechanisms, and influence factors for the synthesis of nanoparticles have also been discussed.

**Silver nanoparticles**

Silver nanoparticles (AgNPs), an important broad-spectrum antimicrobial agent, have been extensively applied in biomedical fields and as consumer products (Guo et al., 2015). The related properties, applications, and characterization methods of AgNPs can be obtained from the comprehensive presentations (Wei et al., 2015; Huang et al., 2017; Durán et al., 2011). Several research groups have reported that AgNPs could be successfully prepared with WRF. In 2006, Vigneshwaran et al. reported that the stable AgNPs could be obtained by using *P. chrysosporium* (MTCC 787) as a platform for the bioreduction of silver nitrate. In this study, they found that silver ions (Ag+) were firstly adsorbed on the mycelial surface through the interactions with the chemical functional groups such as carboxylate anion, carboxyl and peptide bond of proteins, then the reduction process was held by reducing sugar from the saccharides on the mycelia. It was believed that protein acted as capping agent was responsible for the stabilization of AgNPs. AgNPs were distributed uniformly on the surface of the fungal mycelia as shown in Figure.3 (Vigneshwaranet al., 2006). When *T.versicolor* challengedwith silver nitrate solution, this fungus exhibited the similar reduction process to synthesize AgNPs, and the presence of protein was also confirmed as stabilizing and capping agent surrounding AgNPs (Sanghi and Verma, 2009b). Moreover, this study reported that the size of AgNPs produced in media (25-75 nm) was lower than that on mycelium (441-495 nm), which suggested that the synthesize mode should be selected for preparing the different sizes of nanoparticles. Additionally, the alkaline condition was necessary for shortening reduction reaction time, during the reduction process, the color of media solution changed from colorless to light pink, reddish brown and finally to dark brown within 60 min. However, the color turned light brown in 48 h under normal condition. The results were similar with the report using *Pleurotus sajor caju* fungus as bioreduction agent by Nithya and Ragunathan ([2009](#OLE_LINK27)). Furthermore, the intermediates were detected. Ag2S was formed on the surface of mycelium due to the surface S–H groups of the fungus played a major role in reduction process, whereas the Ag2O predominated in the media with the presence of glucose and dissolved oxygen (Sanghi and Verma, 2009b). This phenomenon of producing different intermediates should be further studied for the thorough understanding of extracellular and intracellular reduction process and mechanism.

To obtain more species of WRF that can be used in AgNPs biosynthesis, in 2013, five species of WRF (*Schizophyllum commune*, *Pycnoporus sanguineus*, *Lentinus sajor caju*, *Trametes feei*, and *Trametes pocas*) were isolated from the Malaysian rainforest to examine their capabilities to synthesize AgNPs. After testing, *P. sanguineus* and *S. commune* were found to possess the capacity of synthesizing AgNPs with an average particle size range from 52.8 to 103.3 nm, and the yield of AgNPs produced by *P. sanguineus* was the highest. They also found that high concentration of secreted protein would enhance the production of smaller AgNPs. The different bioreduction modes of AgNPs were also investigated in culture supernatant with fungi-secreted proteins (CS), on the mycelia pellet (MP), and in the silver nitrate solution with the released NPs from mycelia pellet (SN), respectively. The results showed that the AgNPs with different particle sizes and polydispersity could be synthesized through the three modes of extracellular (SN), cultural-free supernatant (CS), and intracellular (MP). They also suggested that extracellular synthesis is more promising over intracellular synthesis through the structural and morphology characterizations analysis, because the formed AgNPs tended to agglomerate and were trapped in the fungi mycelia. Moreover, the antimicrobial activity of AgNPs produced in the SN was more effective than those in CS and MP, which was the indication for the design of suitable particle size of AgNPs for biomedical and biopharmaceutical fields ([Chan and Mat, 2013](#OLE_LINK28)).

Overall, the synthesis of AgNPs by WRF can be concluded into three aspects: 1) reaction process: Ag+ adsorbed on mycelial surface through the interactions with chemical functional groups and reduced by the bioreducing agent such as reducing sugars and protein; 2) reaction region: extracellular and intracellular; 3) influence factors: pH, culturing time, media composition. However, the biosynthesis of nanoparticles by intracellular mode makes downstream processing difficult and limits the development of this green procedure, because the additional operation like sonication is required to separate the nanoparticles trapped in mycelial surface or formed in biomass (Sanghi et al., 2011). Now that the extracellular mode plays a key role in the biosynthesis as well, thus the development of extracellular process is reasonable and practicable from the application point of view.

**Gold nanoparticles**

The mycelium-free extract of WRF is effective to biosynthesize nanoparticles. However, it is unclear that how the extracellular enzymes secreted by WRF function in the biosynthesis process. To understand the role of extracellular enzymes in biosynthesis, an in-depth study should be carried out. Sanghi et al. ([2011](#OLE_LINK29)) explored the main enzyme in the case of *P. chrysosporium* media for the synthesis of extracellular gold nanoparticles (GNPs). When the growth medium (GM) was exposed to tetrachloroauric acid (HAuCl4) solution at room temperature, there was no color change for long time, whereas the color changed with time from colorless to light orange, light purple and finally dark or vivid purple in 35 min at 37 ˚C, and 1 h at 45 ˚C, indicating that reaction temperature had a great effect on the particle formation. Through the UV–visible spectrum analysis, the proteins were found to be utilized and responsible for the extracellular formation of GNPs. The production of GNPs was higher in 7-day-old age fungus GM than that by 5-day-old and 10-day-old cultures due to the higher secreted protein yield. The enzyme analysis showed that laccase was the dominating enzyme in case of GM and acted as a reducing agent. Nevertheless, the role of pure enzyme in synthesis of nanoparticles still remains obscure.

As almost all species of WRF can produce laccase, and the enzyme in its active form was actually catalyzing oxidation not reduction ([Hatakka, 1994](#OLE_LINK30)). Thus, it is necessary to understand how the bioreduction occurs in the pure enzyme solution. Working towards this goal to understand the role of enzyme in the synthesis of nanoparticles, El-Batal et al. ([2014](#OLE_LINK31)) carried out a guiding experiment on GNPs biosynthesis with laccase. In this work, the laccase obtained from *Pleurotus ostreatus* using solid state fermentation was studied. They found that the concentration of GNPs increased with the increasing temperature from 40 to 100 ˚C in the presence of laccase. The temperature effect on the synthesis of GNPs was similar with the previous study ([Faramarzi and Forootanfar, 2011](#OLE_LINK32)). In this case, the optimum temperature of enzyme activity of laccase was between 30–50 ˚C, and the enzyme activity rapidly lost at temperature above 60 ˚C. However, the highest productivity of GNPs was observed at 100 ˚C, at which the enzyme activity and structure have been destroyed, indicating that laccase possibly performed the reduction process as a protein not as an active enzyme. Thus, further research should be carried out to explain the detail reduction process. Likewise, these proteins containing negatively charged carboxylic groups will stabilize the GNPs through the creation of repulsive forces. The characterization analysis of GNPs further suggested that enzyme protein act as reducing agent to synthesize and stabilize GNPs (El-Batal et al., 2014). It suggested that the enzyme in the extracellular act as the reducing agent, then the reducing substances which can act as reducing agent, such as polysaccharide, should be explored further. Besides laccase, LiP and MnP are also the important enzymes in WRF, their roles in synthesis of nanoparticles need to be studied as well.

**Other nanoparticles**

The biosynthesis of copper/copper oxide nanoparticles with the mycelium-free extract of WRF, *Stereum hirsutum*, was investigated under different pH conditions and three different copper salts (CuCl2, CuSO4, and Cu(NO3)2)(Cuevas et al., 2015). The analysis of the UV-visible spectra showed that the absorbance values of maximum surface plasmon peak increased with the increasing of pH regardless of copper salts. However, at the same pH condition, the absorbance value was highest in the presence of CuCl2, indicating that the CuCl2 and neutral or basic conditions were more effective for *S. hirsutum* to biosynthesize copper nanoparticles. In addition, in the presence of CuCl2, the maximum surface plasmon peak at 620 nm was observed when the pH values were 7.0 and 9.0, while at 670 nm for pH 5.0. The pH conditions could not change the maximum surface plasmon peak position (at 670 nm), which indicated the formation of cupric oxide (CuO) nanoparticles, in the presence of CuSO4 and Cu(NO3)2. The nanoparticles characterization analysis of the copper nanoparticles biosynthesized using CuCl2 suggested that the release of extracellular protein by WRF result in the formation and stabilization of biosynthesized nanoparticles (Cu0, Cu2O, and CuO nanocrystals). Meanwhile, the surface of nanoparticles is surrounded by a biopolymer likely polycarbohydrate, which is in agreement with the aforementioned biosynthesized AgNPs. *P. chrysosporium* was found to be capable of synthesizing elemental selenium from selenite but not from selenate, and the possible glutathione-dependent mechanism was involved in the reduction of selenite. The intracellular formation mode was dominated due to the fact that majority of selenium nanoparticles were distributed inside the fungal cells and some localized with the fungal cell walls (Espinosaortizet al., 2015). As a typical semiconductor material, cadmium sulfide (CdS) nanopartcles also can be synthesized by the reduction of toxic Cd using fungus *T. versicolor* in continuous column mode. In this experiment, thiol groups of the fungal protein played critical roles in the detoxification of Cd and in the production of highly stable and autocapped CdS nanoparticles (Sanghi and Verma, 2009a). Likewise, Chen et al. (2014b) reported that the CdS quantum dots (QDs) could be synthesized by *P. chrysosporium*. In the study, they found that the increasing pH enhanced the production of CdS QDs, and the nanoparticles were adsorbed on the mycelial surface, the release of active sulfur-containing substances such as cysteines may play an important role in the process of synthesis. Moreover, White rot fungus *Coprinellus sp* could produce Mn oxide nanoparticles by the oxidation Mn(II) with MnP enzyme (Droz et al., 2015).

All these reports show that WRF have the potential in both biosynthesis and bioremediation. For special nanoparticle biosynthesis by WRF, there are several conditions that should be taken into consideration, such as the percursor compound concentration and type, the media solution pH, temperature, etc. Although different WRF may have the similar biosynthesis process (adsorbtion/reduction) of nanoparticles as shown in Figure.4, the different synthesis mechanisms may be involved in the fabrication of different nanoparticles. Some synthesis mechanisms on nanoparticles using other fungi have been proposed. For example, NADH-dependent reductase was responsible for the reduction of Ag+ using *Aspergillus terreus* (Li et al., 2012); the cofactor NADH and nitrate reductase enzyme might account for the synthesis of AgNPs using *Fusarium acuminatum* (Ingleet al., 2008), which are not elucidated in the current reports of nanoparticles synthesis by WRF. However, the actual mechanism of biosynthesis of nanoparticles by WRF is still not known. Thus, considerable efforts should be paid to study the synthesis mechanisms under different conditions for obtaining appropriate nanoparticle for special field.

**The response of white rot fungi exposure to nanoparticles**

Although nanoparticles can improve the bioremediation performance of WRF, their potential damage on WRF will occur in the presence of nanoparticles (Guo et al., 2015; Huang et al., 2017), which will cause the decrease of bioremediation capacity of WRF. Thus, it is imperative to understand the response of WRF to nanoparticles for the development of biotechnology and nanotechnology.

Hitherto, many studies have verified the inhibitory impacts of nanoparticles on WRF indirectly. For instance, Cu, Zn, B, and Ag nanoparticles showed the inhibitory effect on the white-rot test fungus *T. versicolor* (Kartal et al., 2009). Similarly, during exposure to *T. versicolor*,the wood without treatment showed chemical changes, while no significant changes were observed in the wood structural components after being impregnated with aqueous dispersion containing Ag, Cu and ZnO nanoparticles (Akhtari et al., 2013). Both Ag and Cu nanoparticles could inhibit the growth of *T. versicolor* hyphae significantly. Ag nanoparticles inhibited the growth of *T. versicolor* at a higher content level, in contrast, Cu nanoparticles exhibited more conspicuous antifungal effect at lower content (Taghiyari et al., 2014). Importantly, CuO and SnO2 nanoparticles were found to inhibit the decay by *T. versicolor* in both weathered and unweathered specimens (Terzi et al., 2016). TiO2 nanoparticles also could prevent fungal colonization in wood (Filpo et al., 2013). Indeed, various nanoparticles have showed the inhibitory effect on the growth of WRF from the view of the wood protection. However, the knowledge on the mode of action of WRF when exposed to nanoparticles was limited.

With the different chemical compositions of various nanoparticles, the fungi will exhibit different sensitivities and responses during exposure to different nanoparticles as reported by Galindo et al. (2013). In their study, they found that the tested nanoparticles could not only inhibit the fungi growth, but also induce changes in chemical composition of fungal mycelium such as protein and polysaccharides. An experiment regarding the change of soluble protein and electrophoretic pattern of *P. chrysosporium* fungus was carried out after exposure to different CoFe2O4 nanoparticles concentrations for 7 days and 14 days. The protein content of fungus mycelium increased slightly with the increasing introduction of CoFe2O4 nanoparticles in medium at 7 days after culture, which was lower than that of at corresponding 14 days after culture. However, there was a slight decrease in protein content when compared with the treatment without CoFe2O4 nanoparticles after 14 days culture (Oprica and Ungureanu, 2015). Shah et al. (2010) elucidated the toxicity action of copper and iron nanoparticles against *T. versicolor*. They found that the production of cellulose degrading enzymes (β-glucosidase, β-xylosidase and cellobiohydrolase) was significantly decreased when exposure to both nanoparticles. The possible mechanisms were proposed as two aspects: 1) the nanoparticles that be bound to cell wall of fungi may interfere with the enzyme secretion or gene expression of enzymes; 2) the generation of oxygen radicals after the interreaction will result in the oxidative stress on the cell, thus causing the change of enzyme production. Recently, the toxicity effects of carbonaceous materials on WRF have been reported. Berry et al. (2014) reported that the enzyme production of WRF was altered and the oxidative enzymatic response of WRF to single-walled carbon nanotubes was complex, which might be mediated by various factors such as fungi species and media compositions. Xie et al. (2016) found that graphene oxide could inhibit the growth of *P. chrysosporium* and induce the morphology changes, ultrastructure disruption, and decomposition activity loss. Thus, the toxictity of nanoparticles on WRF is common issue. However, the knowledge on the actual response of WRF to nanoaprticles is limited. For obtaining more comprehensive information on the biological resistance, properties of nanoparticles (such as morphology and size) and the physiological effects of WRF (such as oxidative enzymatic response and structure change) should be taken into account.

**Conclusions and** **Challenges**

This mini-review aims at stating the current status of research on the application of WRF that relates to nanoparticles. It points to the fact that the bioremediation performance of WRF or enzymes will be enhanced due to their immobilization on nanoparticles, and the great potential applications of WRF for the biosynthesis of metallic nanoparticles. Moreover, the ecotoxicity of nanoparticles on WRF are presented as well. However, the research on the application or interaction of WRF and nanoparticles is still at an early stage. Thus, it claims that a massive work should be carried out to understand the potential application of WRF and nanoparticles. Considerable challenges will limit the development of this important enviromental microorganisms and nanoparticles, four such challenges are presented as follows:

1. For bioremediation, although the immobilization can enhance the wastewater treatment efficiency, the applications of immobilized WRF are still confined to laboratory research. Usually, several pollutants may coexist in wastewater that will increase the difficulty of treatment, thereby obtaining more stable support for WRF is neccessary. Moreover, various modified or doped nanoparticles should be prepared and designed for improving the bioremediation. In addition, although the presence of nanoparticles can enhance the bioremediation performance of WRF at certain extent, the toxicity of nanoparticles should also be noted.
2. Depending on the synthesis conditions and selected WRF types, the sizes of biosynthesized nanoparticles can vary considerably, which will impact the properties and applications of nanoparticles (Ray, 2010; Dal Lago et al., 2011). However, the size-selected nanoparticles biosynthesis is difficult due to the complex synthesis condition and follow-up treatment. It has reported that the morphology is a critical characteristic for nanoparticles (Ray, 2010). The nanoparticles with various shapes can be produced by plants and plant-derived materials (Iravani, 2011), and shape of gold nanoparticles (cubic and octahedral) could be controlled by using *Plectonema boryanum* UTEX 485 with different precursor aqueous solutions (Au(S2O3)23- and AuCl4- ) by tuning the reaction temperature and time (Lengke et al., 2006), while merely the spherical nanoparticles were obtained using WRF as biological resource (Table 1). Therefore, a more arduous work on improving the production procedures is required.
3. Separation of produced nanoparticles is an important issue for further applications. The extraction of nanoparticles from WRF mycelia or media is not well investigated. Various operation approaches have been used for the separation of nanoparticles, however, these processes may change the structure and properties of nanoparticles (Iravani, 2011). Compared to intracellular production, extracellular production makes the process simpler to obtain the nanoparticles (Bhainsa and D’Souza, 2006). Thus, it is the key to ensure the high yield of extracellular substances for biosynthesis and understand the biochemical mechanisms of the synthesis of nanoparticles to better control their size and polydispersity.
4. Owing to the presence of biological resistance, further analysis need to be performed in order to determine whether the toxicity effects on WRF occur druing the process of nanoparticles biosynthesis. For biological resistance, the detail response mechanisms should be probed via intracellular and extracellular behaviors of nanoparticles. Since the WRF and nanoparticles have been widely applied in various fields, having a knowledge of their roles, behaviors, interactions is important for the development of biotechnology and nanotechnology.

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Compliance with ethical standards

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

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**Table**

Table 1. White rot fungi in the biosynthesis of various metallic nanoparticles

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| White rot fungi | Nanoparticle | Shape  | Size (nm) | Refences |
| *Phaenerochaete chrysosporium* | Ag | pyramidal | 50-200  | Vigneshwaranet al., (2006) |
| *Trametes versicolor* | Ag | spherical | 25-491  | Sanghi and Verma, (2009b) |
| *Pleurotus sajor caju* | Ag | spherical | 5-50  | Nithya and Ragunathan, (2009) |
| *Pycnoporus sanguineus* | Ag | spherical | 52.8-70.2  | Chan and Mat, (2013) |
| *Schizophyllum commune* | 53.9-103.3  |
| *Phaenerochaete chrysosporium* | Au | spherical | 10-100  | [Sanghi et al., (2011](#OLE_LINK29)) |
| *Pleurotus ostreatus* | Au | — | — | [El-Batal et al., (2014](#OLE_LINK31)) |
| *Stereum hirsutum* | Cu | spherical | 5-20  | [Cuevas et al., (2015](#OLE_LINK33)) |
| *Phaenerochaete chrysosporium* | Se | spherical | 35-400  | Espinosaortizet al., (2015) |
| *Trametes versicolor* | CdS | spherical | 8-15  | Sanghi and Verma, (2009a) |
| *Phaenerochaete chrysosporium* | CdS | spherical | 1.5-2.0  | Chenet al*.,* ([2014](#OLE_LINK35)b) |

**Figure Captions**

Figure.1. The proposed mechanism of phenol degradation. Reprinted with permission from Ref Huang et al., (2015b).

Figure.2. The proposed mechanism for γ-Fe2O3-facilitated biodegradation of BPA by white rot fungus. Reprinted with permission from Ref Li and Zhang, (2016).

Figure.3. ESEM-EDX analysis of fungal mycelium challenged with silver nitrate. (a) Micrograph recorded on the surface of the fungal mycelium. (b) The EDX spectrum for the nanoparticles visualized on the surface of fungal mycelium. Reprinted with permission from Ref Vigneshwaranet al., (2006).

Figure.4. Proposed mode of nanoparticles biosynthesis by white rot fungi: (A) electrostatic interaction between metal ion and chemical functional groups in the cell wall; (B) reduction of Mn+ to M0 state by the reduction agent; (C) intracellular biosynthesis of nanoparticles, and (D) extracellular biosynthesis of nanoparticles. Adapted from Ref [Dhillon et al., (2012](#OLE_LINK24)).

Figure.1.



Figure.2.



Figure.3.



Figure.4.

