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# Review on the Bioremediation by *Aspergillus niger*

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***Aspergillus niger* (*A. niger*) is one of the most important microorganisms used in biotechnology. It has been in use already for many decades to produce extracellular (food) enzymes and citric acid. In addition, *A. niger* is used for biotransformations and waste treatment in the last two decades. This review summarizes available information on *A. Niger* derived biomass for bioremediation of different types of pollutants include metals, radionuclides, pesticides, phenols and dyes. It is clear that *A. niger can* be used to prepare biomass, that can be applied in various aqueous treatment processes, namely to remove organic pollutants, dyes, organic compounds, radionuclides and heavy metals.**

**Key words:** *Aspergillus niger*, Bioremediation, biomass.

Bioremediation refers to the use of microorganisms to eliminate or reduce the concentrations of hazardous wastes at a contaminated site[1]. Bioremediation is often considered a cost effective and environmental friendly method and is gradually makingin roads for environmental clean-up applications. Bioremediation relies on improved detoxification and degradation of toxic pollutants either through intracellular accumulation or via enzymatic transformation to less ornon-toxic compounds[2]. Many microorganisms naturally possess the ability to degrade, transform, or chelate various toxic chemicals. Fungi possess the biochemical and ecological capacity to degrade environmental organic chemicals and to decrease the risk associated with metals, metalloids and radionuclides, either by chemical modification or by influencing chemical bioavailability.

*A.niger* is a dark colored filamentous fungus belonging to phylum Ascomycota (Fig. 1). *A. niger* has been the subject of research and industrial use for several decades. It first acquired practical importance in 1919, when its ability to produce citric acid was industrially exploited. Since the 1960s, *A. niger* has become one of the most important industrial microorganisms and produces a variety of enzymes such as cellulase and xylanase[3], phytases[4], amylases[5,6], lipase[7] and peptidases[8]. Enzymes from *A. niger* have been used in food production for several decades, and even though their peptidases have been studied[9]

*A. niger* plays a significant role in the global carbon cycle, As a common member of the microbial communities found in soils. Moreover, *A. niger* has a remarkably versatile metabolism, enabling growth on a wide range of substrates under various environmental conditions. Its ability to degrade a range of xenobiotics through various oxidation, hydroxylation, and demethylation reactions provides immense potential for use in bioremediation[10-12].

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Fungi are recognized for their superior ability to produce a large variety of extra cellular proteins, organic acids, enzymes and other metabolites, and their waste biomass may be used as effective biosorbent for removal, reduction and detoxification of industrial effluents ingredients[13]. The analysis of cell-wall composition of *A.niger* biomass has been studied by Johnston, IR, 1965[14]. Sugars such as, glucose, galactose, mannose, arabinose, glucosamine and galactosamine, all in the d-configuration and the small amount of l-galactose have been found in the cell wall analysis. According to Johnston report[14], the main structure of cell wall contains carbohydrate and hexosamine and small amounts of lipid and protein.

Large amounts of biomass are produced during the citric acid fermentation process and finding the best way for its consumption is well worth considering. Fungal biomass seems to be a very efficient and inexpensive sorbent that can be produced at low cost by using simple fermentation techniques and cheap growth media. It is also available as a by-product or waste material from various industrial processes[15]. The aim of this study is the investigation of some applications of *A. niger* biomass.

**Heavy Metals and radionuclides**

Wastewater treatment containing heavy metal ions is one of the major concerns in environmental cleaning. There are various treatment methods, e.g. chemical precipitation, evaporation, coagulation–flocculation, flotation, ion exchange, membrane filtration, and adsorption that can be employed to remove heavy metals from contaminated wastewater[16]. All these conventional methods have some advantages and disadvantages such as high consumption of reagent and energy, low selectivity, high operational cost and generation of secondary pollutants[17]. Therefore, environmental and public health engineers have been searching for an inexpensive and efficient treatment for metal-containing wastewaters. Nowaday's use of renewable resources like microbes and plants (bioremediation and phytoremediation) to tackle heavy metal problems is a feasible and useful treatment method leading to detoxification of industrial effluents and wastewaters[18]. Recently, the use of some fungal biomasses for metal elimination has been investigated by some studies[19-21]. The use of dead biomass appears to be more attractive as compared with live biomass, since heavy metals or other toxic pollutants have few influences on the biosorption process. Besides, there is no requirement for the supplement with any nutrients for maintaining growth of dead biomass[22]. The cell walls of these types of biosorbents containing polysaccharides

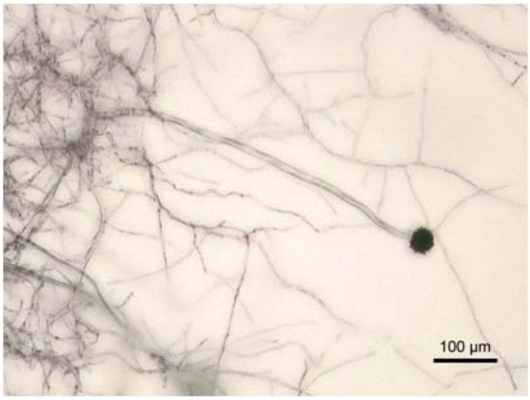


**Fig. 1.** Micrograph of *A. niger*

**Table 1.** Bioremediation of Heavy Metals and radionuclides using *A. niger* biomass

| Heavy Metals and radionuclides | References |
|---|---|
| Lead, zinc, cadmium, chromium, copper, nickel | [30, 35] |
| Cobalt, lead, cadmium, chromium, copper, nickel | [36] |
| Copper, manganese, zinc, nickel, iron, lead, cadmium | [37] |
| Copper and zinc | [38] |
| Copper, lead | [39] |
| Lead, copper | [40] |
| Zinc | [41, 42] |
| Nickel | [43] |
| Copper | [44] |
| Chromium | [31, 45, 46] |
| Uranium | [32] |
| Thorium | [33] |
| Radioactive cobalt | [34] |

**Table 2.** Bioremediation of organics, pesticides and PAH using *A. niger* biomass

| | |
|---|---|
| Phenol | [50] |
| Pentachlorophenol | [49] |
| Endosulfan | [48, 54] |
| Phenanthrene | [47, 59] |
| Chrysene and benzo(a)pyrene | [55] |

and proteins with different functional groups such as amine, carboxyl, hydroxyl, sulfates, and phosphates responsible for interaction with metal ions[22].

*A. niger* has been used to remove metals from the environment by either adsorption of the metals to fungal cell wall components, or complexation of the metals with organic acids produced by the fungus as shown in Table 1 .The fungus has been used commercially in the production of organic acids, such as citric acid[23], oxalic acid[24] and gluconic acid[25]. These acids are well-known lixiviants for the leaching of heavy metals from solid wastes[26], and may reduce the cost of commercial heavy metal decontamination and decrease any environmental impacts resulting from metal contamination[27]. Metal leaching by heterotrophic micro-organisms generally involves an indirect process with microbial production of amino acids, other organic acids, and other metabolites. These metabolites dissolve metals from minerals by displacement of metal ion from the ore or soil matrix by hydrogen ions, or by the formation of soluble metal complexes and chelates[28]. *A. niger* has been found to overproduce organic acids that can serve as leaching agents for the solubilisation of Al, Fe, Mn, Ni, Pb, Cd, Cu,and Zn from fly ash[29]. *A. niger* was used for the leaching of heavy metals from contaminated soils[28]. *A.niger* are used for chromium biosorption through ion exchange mechanism[30]. *A. niger* strain, isolated from soil of leather tanning effluent, has been found to remove chromium very effectively from liquid medium as well as from soil microcosm with different concentrations of chromate[31]. The ability of *A. niger* biomass to bind and remove heavy metals, i.e. Ni, Pb, Cr from real wastewater was investigated. Also A, Niger was used for the removal of radionuclides such as uranium, and thorium[32, 33]. Bioremediation of $^{60}$Co from simulated spent decontamination solutions by *A. niger* was reported[34].

**Organics**

In modern society, an increasing number of hazardous organic compounds are being discharged into the environment. Most are degraded or detoxificated by physical, chemical and biological treatments before released into the environment. Although the biological treatments are a removal process for some organic compounds, their products of biodegradation may also be hazardous. Moreover, some nondegradable compounds discharged into the environment along with the treated compounds can cause problems because they usually come back to human beings through the several channels such as bioaccumulation. As a result, organic molecules that are not biodegradable, can still be removed from the wastewater by the microbial biomass via the process of biosorption. Biosorption is also becoming a promising alternative to replace or supplement the present removal processes of organic pollutants from wastewaters. Among these pollutants, phenolics and pesticides have recently been of great concern because of the extreme toxicity and/or persistency in the environment. Biosorption of these type of hazardous organics by selected live and dead microorganisms has been investigated by various workers[47-50].

Phenol is an important toxic compound listed as a priority pollutant by the EPA and other environmental protection agencies because of its high toxicity and possible accumulation in the environment. Mathialagan and Viraraghavan

**Table 3.** Bioremediation of Dyes using *A. niger* Biomass

| Dyes | |
|---|---|
| Acid Black 1 | [70] |
| Acid Red, Orange II, | [71] |
| Acid Blue 2 | [68] |
| Acid Blue 29 | [68] |
| basic Blue 9 | [72] |
| Basic Blue Acid Blue Congo Red | |
| Disperse Red 1 | [72, 73] |
| Ci direct blue 199 | [66] |
| Congo Red | [49, 69] |
| Direct Violet, | |
| Direct Brown and Direct Green | [74] |
| Direct Blue 199 | [66] |
| Gryfalan black | [75] |
| Orange II | [76] |
| Polar Red | [77] |
| Reactive black 8 | [63] |
| Reactive blue 140 | [65] |
| Reactive Brilliant Red | [52] |
| Scarlet Red; Greenish Blue; Brilliant | |
| Violet | [78] |
| Sulfur black, acid red 151, dimarene | |
| blue K2RL, Orange II Synazo | [53] |
| yellow HF2GR | [64] |

studied the biosorption of pentachlorophenol (PCP) on treated *A.niger* biomass. They pretreated live fungal pellicles in five different ways: autoclaving, treatment with 0.1M $HNO_3$, treatment with 0.1M $H_2SO_4$, treatment with 0.1M NaOH, treatment with a laboratory detergent (5 g $l^{-1}$). They found that the biosorption was pH dependent. The removal of PCP decreased with the increase of pH for all types of biomass. They also evaluated the results with different kinetic and isotherm models[51]. They observed that sulphuric acid-treated biomass powder was the most effective for the removal of phenol. Sodium hydroxide pretreated and laboratory detergent pretreated biomasses gave very close removals. Alkali pretreatment has also been shown to be a probable cause for destroying autolytic enzymes that cause putrefaction of the biomass and for removing lipids and proteins that mask reactive sites[50]. Gallagher *et al*.,[52] have also suggested that NaOH pretreatment may also increase the percentage of chitin/chitosan in the whole cell wall fraction by dissolving certain biopolymers from the cell wall. Chitin/chitosan units may then be responsible for the uptake of phenol in the case of NaOH pretreated biomass[53].

**Pesticides**

The chlorinated organic pesticide endosulfan is the major agrochemical responsible for environmental pollution. It enters air, water, and soil environments during its use and manufacturing. Due to its hydrophobic nature, it is also associated with soil. Among different groups of microorganisms, fungi have their own advantages in cultivation, maintenance, and tolerance to pesticides over bacteria, especially for chlorinated pesticides. All of these applications attract scientists to use fungi in the biodegradation process. As per the earlier report of *A.niger* showing good results at broth condition[54] in endosulfan degradation, endosulfan was degraded in just 120 h. Bhalerao (2012) applied strategy of *in situ* bioremediation by bioaugmentation for soil contaminated with organochlorine pesticide endosulfan in an open pot[48]. It showed that previously isolated fungal culture *A. niger* can be effectively used for endosulfan degradation.

Poly Aromatic Hydrocarbons (PAH)

Previous work[55] has reported that *A. niger* was efficient degraders of PAHs in high concentrations compared to other non-ligninolytic fungal strains which produce more toxic intermediaries than the original compound and tolerate lower PAH concentrations, and also compared to ligninolytic fungi not grown in soil and which are displaced by native soil microflora. Niger able to transform a variety of PAHs, including chrysene and benzo(a)pyrene, to polar metabolites[55, 56]. This microorganisms carry out a monooxygenation of the PAH molecules by the intracellular cytochrome P-450 dependent oxidase system[57]. These fungi do not produce extracellular peroxidases, however, they do produce cytochrome P450 monooxygenase which can oxidize PAHs to epoxides and dihydrodiols: highly potent carcinogens that accumulate in soil[58]. Other study in which A niger, grown on sugarcane bagasse and added to a soil spiked with 400 ppm phenanthrene, achieved 54% removal of the pollutant from the soil after 7 days,[59].

**Dyes**

The textile industry is a substantial consumer of water and produces enormous volumes of contaminated water; the most important contaminants are dyes. The complex aromatic structure of the dyes is resistant to light, biological activity, ozone and other degradative environmental conditions. Thus conventional waste water treatment remains ineffective. Also, anionic and nonionic azo dyes release toxic amines due to the reactive cleavage of azo groups[60]. There are various methods for the treatment of textile wastewater for the removal of dye. These broadly fall into three categories: Physical, Chemical and Biological. The major disadvantage of physico-chemical methods has been largely due to the high cost, low efficiency, limitedversatility, interference by other wastewater constituents and the handling of the waste generated. Microbial decolourization have the advantage of being cost-effective and environmentally friendly and producing less sludge. The mechanism of microbial decolouration occurs from adsorption, enzymatic degradation or a combination of both.

*A. niger* is studied more than other biomasses for decolorization of waste water samples (Table 3). The biomass of *A.niger* have been developed for decolorization of waste waters containing, acid blue[61] basic blue 9[49]Congo red[62] reactive black 8 [63] Sulfur black[53], acid red 151, dimarene blue K2RL, orange II [53] yellow HF2GR[64] from textile effluent, reactive blue 140 [65], Ci direct blue199 [66]. Reported results show the efficiency of the *A. niger* biomass for dye removal from wastewaters. Both active and inactive forms of *A. niger* have been applied for afore mentioned researches and investigations. The mechanism of dye adsorption on the surface of fungal biomass is related to electrostatic attraction between negatively charged dyes and positively charged cell walls and this is because of chitin, polysaccharides amino acids and lipids which make the cell wall structure[67].

Gallagher *et al*.[52] investigated three types of fungi, including *Laminaria digitata*, *Rhizopus oryzae*, and *A.niger*, to remove Reactive Brilliant Red, a reactive dye suggesting that adsorption was occurred by combined mechanisms onto a heterogeneous surface[52]. Fu and Virarahavan[68, 69] investigated the removal of Basic Blue 9 (cationic), Acid Blue 29 (anionic), CongoRed (anionic), and Disperse Red 1 (nonionic) dyes from aqueous solutions by biosorption on dead and pretreated *A.niger* fungus. They found that *A. niger* is capable of removing dyes from an aqueous solution. They explored that three major functional groups: carboxyl, amino and phosphate, and the lipid fraction in the biomass of *A.niger* played an important role in the biosorption of these dyes[68, 69].

## CONCLUSION

This review paper highlights the capacity *A. niger* biomass in different pollutants treatment. We overview about using *A. niger* in the removal of different types of pollutants including metals radionuclides, phenols, PAH, pesticides and dyes.

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