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Biomass of *Aspergillus Niger*: Uses and Applications

Mohsen Shahlaei[a] and Alireza Pourhossein[b]*

[a] Novel Drug Delivery Research Center, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah,Iran.
[b] Young Researchers Club, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran.

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Fungal biomass of *Aspergillus Niger*, is a byproduct of citric acid fermentation that has proven to be a valuable biomaterial that can be both beneficial and practical. From an environmental point of view, it can be a useful bioadsorbent to detoxify and decolorize the wastewater samples. In industrial and pharmaceutical applications, it can also be used as an inexpensive and desirable source for commercial production of some expensive and useful biopolymers such as chitin, chitosan and food supplements such as chitin-glucan and glucosamine.

***Corresponding author: Alireza Pourhossein**, **E-mail:** apourhossein@ymail.com

Introduction

Aspergillus Niger is a fungus which is highly used in production of citric acid [1-3]. It can also be used for enzymes production [4]. The enzyme production ability is related to its capacity to utilize varieties of substrates and this is owing to its well-developed enzymatic system.[5]. *Aspergillus Niger* is a dark colored fungus that produces white septate hypha which is profusely branched. Citric acid is commonly produced by a fungus *Aspergillus Niger* using starchy and sugary material [6]. In citric acid fermentation the biomass of *A. Niger* also comes out as a byproduct. By lengthening the process, *Aspergillus Niger* strains biomass growth during citric acid fermentation. This time-increase is mainly for the production of mycelia body of the fungus and its sporulation. The analysis of cell-wall composition of *Aspergillus niger* biomass has been studied by [7]. 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 [7], 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. The aim of this study is the investigation of some applications of *A. Niger* biomass which is produced as a byproduct during the fermentation of citric acid.

Discussion

Dyes removal from wastewaters

The dye removal from industrial wastewaters is one of the most important environmental concerns owing to stability and difficulty of removal by classic methods [8-10]. Some physical, chemical and biological techniques such as oxidation, coagulation, ozonation and adsorption have been widely used for removal of dyes from various wastewaters. Among these methods decolorization by carbon active is the most effective physical process, but it is very costly and the idea of replacing the adsorbent with inexpensive materials is well worth considering. The use of some biomasses and their activated carbons for removal of different kinds of dyes from wastewater samples has increased in recent years. On the other hand the use of microbial biomasses for dye removal has also developed in recent years. The dye removal potential of some microbial biomasses such as Saccharomayses Cervisiae [11] Penicillium [12], Rhizopous [13] Trichoderma Sp [14] Trametes pubescens and Pleurotus Ostreatus [15] Shizophylum commune [16, 17] Alternaria solari [18] have been studied for this purpose in previous studies. Among the fungal biomasses, *A. Niger* is studied more than other biomasses for decolorization of wastewater samples. The biomass of *Aspergillus Niger* have been developed for decolorization of wastewaters containing, acid blue [19] basic blue 9 [20] Congo red [21] reactive black 8 [23] Sulfur black [24], acid red 151, dimarene blue K_2RL, orange II [24] yellow HF2GR [25] textile effluent [25] reactive blue 140 [26], Ci direct blue 199 [27]. 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 aforementioned 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 [13].

Biosorption of Trace elements

The pollution of toxic and trace elements is one of the most important issues in wastewater treatment. Trace metals easily enter the environment by industrial activities and can accumulate in the food chain of humans. Removal of trace elements from wastewaters is important for the protection of the environment and human health. Methods such as ion exchange, neutralization, precipitation, reverse osmosis, membrane separation are commonly applied for detoxification and removal of trace metals from wastewaters. But all of these methods are very costly and involve complex operations. Recently, the use of some fungal biomasses for metal elimination has been investigated [28]. The acetoamide group of chitin, carboxy group of protein, aminophosphate of nucleic acid, amino, amides can make it suitable for formation of metals complex and adsorption of trace elements [28]. The biomass of *Aspergillus Niger* shows good capacity for adsorption of trace metal from wastewaters due to the polysaccharides, proteins and other groups of its cell wall [28]. Because of its high

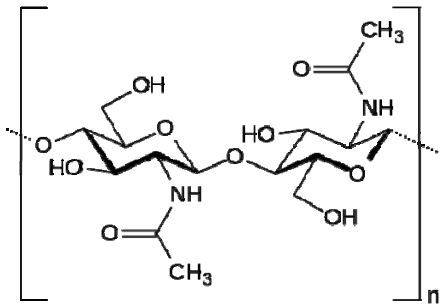
capacity, the biomass of *A. Niger* has been used for adsorption of some trace elements from different wastewater samples [29-32]. Metal removal depends on different parameters such as detoxification period, the concentration of fungal biomass, pH and temperature [33]. Both living and dead biomasses are suitable for metal detoxification but the use of dead biomass is preferable [33]. The removed trace elements with adsorption using *A. niger* biomass have been shown in table 1 [34-52].

Table 1. Trace elements which removed from wastewaters using the biomass of A. Niger.

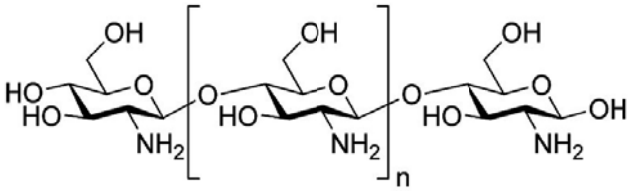
No.	Trace element	references	No.	Trace element	references
1	Silver	[51]	6	Arsenic	[42]
2	Copper	[34-36]	7	Lead	[43-46]
3	Zinc	[34]	8	Chromium	[47-50]
4	Cadmium	[37-40]	9	Nickel	[40]
5	Uranium	[41]	10	Thallium	[52]

Chitin, chitosan and glucosamine production

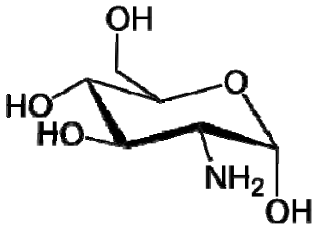
One of the interesting applications of fungal biomass is the use of them for making biopolymers such as chitin and chitosan and their derivatives such as glucosamine. Chitin (Scheme 1) is the second abundant biopolymer in the nature after cellulose. Chitin is the major structural component of the exoskeleton of invertebrates and the cell walls of fungi [53, 54]. Chitin is the source of production of chitosan and glucosamine. Chitosan (Scheme 2) is the n-deacytilated form of chitin and glucosamine is the monomer of chitosan. Chitosan has been identified as having various industrial food and pharmaceutical applications [55-59]. Glucosamine (Scheme 3) is a food supplement that has proven to be very effective in arthritis and joint pain treatments. On the commercial scale crustaceans, shrimp and crab shell waste have been wildly used for production of chitin, chitosan and glucosamine. [60-62]. According to new reports, the use of shellfish glucosamine and chitosan has shown side effects for humans who have shellfish allergies [63]. Another problem with shellfish chitin, chitosan and glucosamine is that some of the shellfish supplies are from seas and oceans. Excessive harvests of shellfish could have negative environmental impacts [63]. The presence of chitin in fungal cell wall makes them the suitable source for production of chitin and its derivatives. Among microbial cell walls, the biomass of *Aspergillus Niger* can be applied for extraction of chitin, chitosan and glucosamine. Extraction of chitosan from the mycelia of *A. Niger* is more cost-effective and less expensive in comparison with crustacean shrimps and crabshells and it has also been reported by other authors [64-66]. The industrial production of chitosan and glucosamine involving the use of biomass of *Aspergillus Niger* has been recently performed by some companies such as Cargill. The chitin percentage in *A. Niger* cell wall has a range between 5–25 percent [66]. Alkali treatment of *A. Niger* biomass using sodium hydroxide for deacytilaton of chitin and protein removal and extraction of chitosan with acetic acid has been applied for chitosan production in previous works [66]. Yumin Du et al. [67] studied the enzymatic method for preparation of chitosan from the waste mycelium of citric acid. Some enzymes such as protease, lysozyme, snailase and novel chitin deacetylase from Scopulariopsis brevicaulis have been applied for chitosan preparation. Decrease in the environmental pollution, and improvement of the quality of the product were achieved by this method. Aggressive Acid treatment of A.niger biomass leads to deacytilaton of chitin in cell wall and Hydrolysis of polysaccharide chain of chitin and forms glucosamine [63]. Reported studies show that *A. Niger* biomass is a good source for safe, non- animal derived, production of glucosamine. It has been validated by the European Food Safety Authority that glucosamine hydrochloride from *Aspergillus Niger* is a safe food ingredient for adult consumption at the proposed intake level of 750 mg of glucosamine per day. Consumers with diabetes or glucose intolerance should be advised to seek medical advice before consumption [68].



Scheme 1. Structure of Chitin



Scheme 2. Structure of Chitosan



Scheme 3. Structure of Glucosamine

Isolation of Chitin glucan (CG)

Chitin-glucan (CG) is a biological copolymer that is composed of chitin macromolecules with covalently linked "D-glucan chains that has many applications in medicine and cosmetics. Some applications are as follow: Reducing of colloid and cloudiness during racking, wine stabilization prior to bottling after fermentation. Elimination of heavy metals such as lead and cadmium, mycotoxins etc. reported in previous works [69]. The CG complex contributes to heart health and protects against cholesterol oxidation which may increase inflammation. Daily supplementation with chitin-glucan reduced oxidized low-density lipoprotein (LDL) cholesterol reduces the risk of atherosclerosis [70]. The biomass of *Aspergillus Niger* is considered as an industrial chitin-glucan source. The alkali-insoluble cell-wall residue of the *Aspergillus Niger* biomass consists mainly of chitin and (1_3, 1_6)-b-D-glucan [71]. Preparation of Chitin–glucan (CG) from the biomass of *A. Niger* was reported in 1979 by Muzzarelli [72]. The method was based on the alkali treatment of biomass with NaOH solution (2.5%) at ambient temperature overnight and then aggressive alkali treatment with concentrated NaOH (40–45%) at130°C for 4–6 h. the procedure, yielded a white powder containing 32% of the polyaminosaccharide and 15–20% glucan. Other methods for CG extraction from the biomass of *A. Niger* involve the alkaline extraction to remove the proteins and alkali-soluble polysaccharides [73-75]. European Food Safety Authority has validated the CG isolated from the biomass of *Aspergillus Niger* as a food supplement [76].

Conclusion

This review highlighted the capacities of *A. Niger* biomass in wastewaters treatment, and also in chitin, chitosan, chitin-glucan and glucosamine production. However, some works on this subject are still laboratory tests and are of less industrial scale application. The dead biomass of fungi is suitable for all uses. Ultimately, the fungal biomasses have great advantages in wastewater treatments and pharmaceutical applications.

Conflict of Interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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