



# Melanin-gamma rays assistants for bismuth oxide nanoparticles synthesis at room temperature for enhancing antimicrobial, and photocatalytic activity



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## ABSTRACT

Melanin pigment has been deemed as a natural photoprotector with strong hydrophobicity. It allured considerable compatibility with many applications in medicine, food, and nanotechnology. *Penicillium chrysogenum* has been devoted to the green synthesis of melanin whereby optimizing its culture and environmental conditions. The impacts of alternative economic L-tyrosine natural sources (unprecedented alternate origins) and gamma radiation were pledged for the potential growing of the pigment. Herein, notable increases in melanin yield (6.4 mg/ml; much higher than nonoptimized one by 40 folds) was obtained by optimizing the culture, and environmental requirements [potato starch (3.0%), yeast extract (5.0%), copper sulfate (0.2 mM), 0.25% L-tyrosine, 0.1% L-glycine, and 0.1% Tween 20 at pH 5.0, and 30 °C for 7.0 days using 180.0 rpm shaking speed]. The addition of banana's peel (2.0%) has been led to increase the melanin production up to (8.3 mg/ml; much higher than optimized one by 1.29 folds). It stimulated the induced enzymes, (i.e., tyrosinase) because it contained significant amounts of L-tyrosine, dopamine, and L-DOPA as resources for melanin biosynthesis. Then irradiated *P. chrysogenum* (2.5 kGy) induced the pigment yield to 10.3 mg/ml; much higher than optimized one by (1.61 folds). On the other hand, we tailored a methodology involved the product of melanin and gamma rays (25.0 kGy) to an eco-friendly synthesis of Bismuth oxide nanoparticles (BiONPs) at the room temperature. Melanin under such alkaline condition functioning as simultaneously hydrolyzes, photo-protection of the Bi seeds, and stabilizer against the uncontrolled growth and the free radicals attack. Whereas the gamma irradiation induced the room temperature condensation reaction to occur, a novel mechanism proposal was discussed. BiONPs were characterized by UV-Vis., DLS, XRD, SEM, EDX, and FTIR. DLS and XRD calculations with TEM analysis exhibited the mean diameter of BiONPs was 29.82 nm. Moreover, the as-prepared BiONPs presented a unique antimicrobial activity against some oral, standard ATCC, and multidrug resistant microbes with ultralow concentrations (0.8 µg/ml). Also, the photocatalytic degradation of Tartrazine dye (TZ), under the UV-Light irradiation, reached 85.0% in 140.0 min. Thereby, owing to its unique characteristics such as cost-effective and scalability method with long-term stabilization, nontoxic nature, excellent chemical inertness, biocompatibility and active properties of BiONPs can find possible purposes in the medical, dental, and cosmetic approaches.

## 1. Introduction

Decades ago the enormous body of research enticed considerable attention to hitherto on the melanin pigment because, it was different irregular blackish brown polymers used as a photoprotective pigment, which mainly exists in microorganisms, plants, and animals [1]. Otherwise, melanins had been used as active polymers in industrial

fields such as food additive [2], cosmetics, and radiation applications [3], nanotechnology [4,5], medicine and other fields. Melanin was used as an antioxidant [6], antitumor agent [7], an antimicrobial tool [5,8], and radioprotective polymer [1]. Dark pigmentation in microorganisms, especially fungal cells contributing to survive against environmental extensions such as oxidizing agents, ultraviolet light, and ionizing radiation. Accordingly, it adapts fungal cells and gives its

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pathogenesis [9].

Fungal melanin is a biomacromolecule polymer that formed by oxidation and polymerization of indolic compounds and/or phenols by the aid of tyrosinase enzyme [10]. Tyrosinase (Oxygen Oxidoreductase) EC 1.14.18.1; is a class III copper protein; furthermore it is an essential enzyme engaging in the process of melanin biosynthesis [11].

L-tyrosine natural sources contain a various amount of carbohydrates, lipids, and amino acids that, participates in microbial pigment production throughout fermentation processes [5]. Among these sources was banana peel that, had a significant role in melanin biosynthesis through introducing L-tyrosin and L-DOPA as melanin resources.

The development of efficient methods for the controlled synthesis of nanocrystals with well-dispersity, stability, and foreseeable morphology is one of the key goals in the advancement of nanoscience. Over the past 20 years, considerable efforts have been devoted to the development of various synthetic courses for preparing well-dispersed nanocrystals [12].

Solution-mediated various synthetic approaches have been considered the most prominent and a feasible method to produce a variety of oxides, whereas it has merged with organic ligands in order to controllably synthesize various shapes and sizes of the targeted nanocrystals [13], which its mechanism is originally postulated by LaMer in 1950 [14]. However, the traditional precipitation approaches for BiOx were comparatively implicated with high manufacturing cost and time-consuming, which initially involves most of them the preparation of precursors like bismuth hydroxide starting from Bismuth nitrate in aqueous solution, then sterically stabilize the obtained seeds with expensive capping agents like cetyltrimethylammonium bromide [15], and dimethylformamide [16], and eventually their thermal decomposition at a temperature as high as 500 °C at a mean time up to 10.0 h.

Besides, for the earliest time to our principles, we established a cost-effective green route to synthesis fine BiONPs at the room temperature. Our method involves the obtained melanin as a stabilizer from the uncontrolled growth, protector from the free radicals attack and gamma irradiation. Meanwhile, the melanin is found to be stable at a high alkalinity which is very preferable to hydrolyze the Bismuth precursor rapidly, then gamma irradiation to our designed solution plays an important role to get rid of the annealing step to induce a room temperature condensation reaction to occur and obtaining BiONPs. A possible mechanism of action was proposed.

Biological synthesis of nanoparticles affords cost-effective and an eco-friendly method. An alternative strategy for the assembly of metal-oxide nanoparticles was applied biomaterials such as plants, and microbial products like metabolites, and pigments as manufactories [17].

Bismuth oxide ( $\text{Bi}_2\text{O}_3$ ) is being a photoactive semiconductor, that has been rendering in the area of catalysis, as a precursor factor in the structure of superconductors, in medical treatments, and in gas sensors [18].  $\text{Bi}_2\text{O}_3$  is a well-popular transition metal oxide [19]. It had been intensively examining due to its exceptional electrical and thermal transportation characteristics [20].

Moreover, BiONPs can provide a high surface area with different electrochemical stability. Recently, BiONPs have brought significant recognition due to their possible uses for sensing zinc in electrochemical sensors [21], viable heterostructured with other metal oxide components for effectively enhancing the electron-hole pair separation [22], and to investigate DNA hybridization [23]. Owing to its unusual features such as nontoxic nature, biocompatibility, and great chemical inertness, BiONPs have been applied as imported immobilizing stands for polyphenol oxidase, and glucose oxidase [24].

Despite the remarkable success of penicillin, now we are selling with pathogenic microbes defiant to several traditional antibiotics [25]. Bacteria and fungi can develop biofilm to increase their significant resistance against antimicrobial agents [26]. Nanoparticles were pos-

sessed large surface areas, and consequently, they were improved intercommunications with living targets [27]. We were confirming the antimicrobial effectiveness of BiONPs for restraining the infection of the oral pathogens, standard ATCC pathogenic bacteria, and multidrug-resistant microbes.

Photocatalytic process necessitated and a hotly debated point was owing to its numerous employment in environmental pollutant dilemma and carbon-free energy production [22]. Photocatalysis is a surface phenomenon that photo-induced redox-ability response due to the separated energetic electron-hole pairs at the surface [28]. Among several metal oxides, BiONPs is a rich earth abundant metal oxide, due to it has a visible a noticeable band gap (ca. 2.8 eV) and strong hole oxidation potential that capable to oxidize and degrade a variety of dyes, liquids, and other substances [29].

Tartrazine dye (TZ) is an azobenzene synthetic golden yellow dye whose construction highlights a trisodium salt from 3-carboxy-5-hydroxy-1 (p-sulfophenyl)-4-(sulfophenyl azo) pyrazolone. It is commonly applied to grinding gum, dye sweets, jams, desserts, syrups, jellies, mustard, drinks, cosmetics, and medicines. As it is a nitrous evolved (azo class), it is degraded in the body to an aromatic amine that is extremely sensitizing. Its principal metabolite recognized to time is sulfanilic acid [30].

TZ had been involved as the feed additive, that maximum is usually effective as allergic responses, should, therefore, be marked with the exact area. Still, urticaria, purpura, asthma, and eczema have been reported, particularly concerning people sensitivity over aspirin [31]. Consequently, the photocatalytic action of the processed photocatalyst (BiONPs) was assessed through the degradation of the original pollutant dyes (TZ) in the aqueous solution following Ultra-Violet lighting as a purpose of irradiation.

In this regard, we have used a classical optimization of the medium compositions and cultivation requirements for advancing the melanin synthesis by *Penicillium chrysogenum*. Exploring the reasonable sources, including L-tyrosine for possible, rising of the melanin concentration. Investigating the influence of various gamma radiation doses on *Penicillium chrysogenum* for emboldening and stimulating of the melanin productions. Synthesis and validation of BiONPs by the co-assistants of both gamma rays and melanin pigment. Examining the antimicrobial potential of BiONPs toward oral pathogens, standard ATCC pathogenic strains, and multidrug-resistant bacteria. Investigation of the photocatalytic degradation of TZ dye using BiONPs.

## 2. Materials and Methods

### 2.1. Chemicals

Media ingredients from (Oxoid) and (Difco). Chemicals and reagents applied in the subsequent investigations, and biological experiments were obtained at analytical standard grade and utilized without further purification.

### 2.2. Microbial Isolates, and Media

In the next study, we selected the abundant fungal isolate examined for melanin pigment production. It created by a screening of remarkable fungal species such as (*Fusarium oxysporum*, *Pleurotus ostreatus*, *Aspergillus niger*, *Trichoderma viridide*, *Penicillium chrysogenum*, *Penicillium citrinum*, *Penicillium brevi-compactum*, *Gliocladium deliguscense* and *Alternaria alternata*) that, recognized in NCRRT [5].

The screening was performed by inoculated the last mentioned fungi (5.0 culture discs, about  $1-3 \times 10^6$  spores/disc) in Vogel medium. It is contained, in gm/L: glucose 20.0,  $\text{NaNO}_3$  15.0,  $\text{K}_2\text{HPO}_4$  2.5,  $\text{CaCl}_2 \cdot \text{H}_2\text{O}$  0.5,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1.0, and distilled water up to 1000 ml, with 0.1 g% L-tyrosine to support the growth of melanin. After that, it was incubated for seven days at 30 °C under shaking condition [32].

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