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Research Paper

Extracellular biosynthesis and characterization of titanium dioxide nanoparticles (TiO_2) by using *Aspergillus* sp. TK4

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Abstract

The broad applications of titanium dioxide (TiO_2) in various fields have drawn attention for the synthesis of TiO_2 nanoparticles. In line with this requirement, there are various studies on the synthesis of TiO_2 nanoparticles. Many chemical and physical methods have been applied to prepare nanoparticles, but synthesis methods utilizing biological materials (e.g., bacteria, fungi, enzymes, and plant extracts) can be considered as eco-friendly alternatives when compared to chemical and physical techniques. The present study was designated to biotechnologically synthesize TiO_2 nanoparticles from titanium(IV) oxide by *Aspergillus* sp. TK4. TiO_2 nanoparticles were successfully synthesized extracellularly from the precursor and characterized by using SEM and EDX techniques. According to the results of SEM and EDX, the nanoparticles obtained are generally distributed between 50-100 nanometers and are composed of titanium and oxygen elements. Besides, it has been observed that the dimensions of some formations can reach 200 nanometers. In conclusion, taking into account the industrial uses as well as the uses in medical fields, the production of TiO_2 nanoparticles with biotechnological means using natural sources is a more advantageous option. Moreover, the results of this study contribute to the development of new biological methods that can be used in the synthesis of TiO_2 nanoparticles.

Keywords: Biotechnological synthesis; *Aspergillus* sp.; TiO_2 nanoparticle.

1. Introduction

Nanotechnology is emerging as a rapidly growing field with its application in science and technology to manufacture new materials at the nanoscale [1, 2]. Recently, nanotechnology has gained extensive applications in the fields of biotechnology. Biotechnological synthesis of nanomaterials has introduced a new approach in the field of nanotechnology [3, 4]. Amongst nanomaterials, a majority of the research has mainly focused on nanoparticles as they have been a wide variety of potential applications in medicine, biology, physics, chemistry, and material science [5, 6].

Besides, nanoparticles are the most noticeable because they have unique properties than the bulk materials. Between the various metal oxide nanoparticles, titanium dioxide (TiO_2) nanoparticles possess interesting optical, dielectric, antimicrobial, antibacterial, chemical stability, and catalytic properties which leads to industrial applications such as pigment, fillers, catalyst supports, and photocatalyst [7-11]. Beside mentioned use areas, TiO_2 nanoparticles are also used in areas related to human health such as bone tissue engineering as well as in pharmaceutical industries because of their non-toxic and biocompatible properties [12-14]. The flexibility in usage of TiO_2 nanoparticles attracts the attention for industrial scale synthesis of these nanoparticles.

Traditionally, most of the nanoparticles were routinely synthesized by various chemical and physical methods. The chemical and physical synthesis of nanoparticles is over costing and often include the use of toxic, hazardous chemicals which may pose environmental risks [15]. For these reasons, there is a growing need to develop simple, low-cost, eco-friendly and bio-based approaches for the synthesis of metal nanoparticles. In this regard, biotechnological methods (use of microorganisms, plants and their derivatives in the synthesis of nanoparticles) are considered more advantageous than conventional methods since they are

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cost-effective and do not require the use of toxic chemicals, high pressure, energy and temperatures [16]. In this regard; because it is easily accessible, it can be reproduced easily in a laboratory environment and has a reducing enzyme for metal ions, a fungus type has been chosen as a microorganism.

Thus, TiO_2 nanoparticles were synthesized through biotechnological methods by using *Aspergillus* sp. TK4 from Ti-based precursors and the obtained products were characterized by using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) techniques.

2. Materials and Methods

2.1. Chemicals and microorganism

Magnesium sulfate heptahydrate (CAS#10034-99-8), potassium phosphate monobasic (CAS#7778-77-0), potassium phosphate dibasic (CAS#7758-11-4), titanium(IV) oxide (CAS#13463-67-7) and potato dextrose agar (110130) were purchased from Merck®; yeast extract (CAS#8013-01-2) and glucose (CAS#50-99-7) from Sigma-Aldrich®.

The fungal strain *Aspergillus* sp. TK4 was provided from the culture collection in the Molecular Biology and Bacteriology Research Laboratory of Biology Department, Ataturk University.

2.2. Synthesis and characterization of TiO_2 nanoparticles

Synthesis of TiO_2 nanoparticles was done according to the protocol previously described by Rajakumar et al. [17] with some minor modifications. Briefly in this procedure, to prepare biomass for biosynthesis studies, the fungus was grown aerobically in a liquid media containing (g/l) KH_2PO_4, 7.0; K_2HPO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.1; $(NH_4)_2SO_4$, 1.0; yeast extract, 0.6; and glucose, 10.0. Then, the final pH was adjusted to 6.2 ± 0.2. The flask was incubated in the orbital shaker at 200 rpm at 37 °C for 15 days. Following of incubation period, the mycelium and extracellular fluid were separated by Whatman filter paper No:1 and the extracellular fluid stored in an amber glass bottle at 4 °C. Then, 100 ml 1mM TiO_2 precursor was added into 100 ml of extracellular fluid and the mixture was incubated at 37 °C for 72 h at 200 rpm. From the beginning of the process, distinct coalescent white deposition starts to appear at the bottom of the bottle, indicating the initiation of transformation. By the end of the synthesis process, the product was centrifuged, filtered, washed with deionized water, and finally dried at 70 °C in a hot air oven for 4h.

Characterization of produced TiO_2 nanoparticles was surveyed with SEM and EDX techniques.

3. Results and Discussion

Visual examinations of the cultures confirmed the synthesis of TiO_2 nanoparticles, in which the color of the cultivation media was changed from colorless transparent to a blurred pale white color during the reaction and finally distinct coalescent white deposition was observed at the bottom of the culture bottle, indicating the synthesis of TiO_2 nanoparticles.

SEM was employed to analyze the structure and size of nanoparticles that were formed. SEM result has shown the formation of individual TiO_2 nanoparticles as well as some aggregates. Mostly, the size of nanoparticles synthesized from precursor was ranged from 50 to 100 nm (Figure 1).

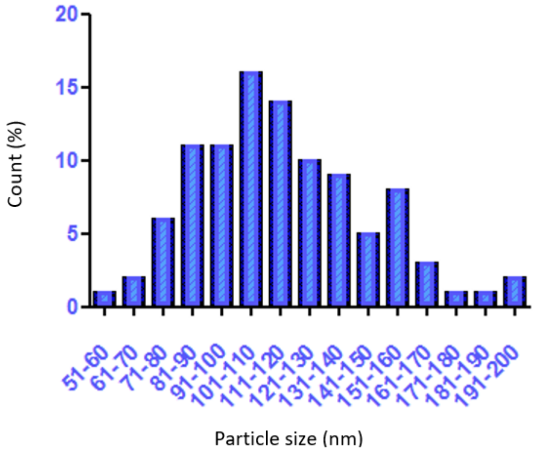

Figure 1. Frequency histogram of synthesized TiO_2 nanoparticles distributions

In addition to this average size distribution, larger and smaller nanostructures have also been observed. SEM image in Figure 2 shows that all the synthesized nanoparticles are spherical-shaped. This result coincides with results already reported, which shows the formation of spherical-shaped nanoparticles and aggregated molecules in Rajakumar et al. [17].

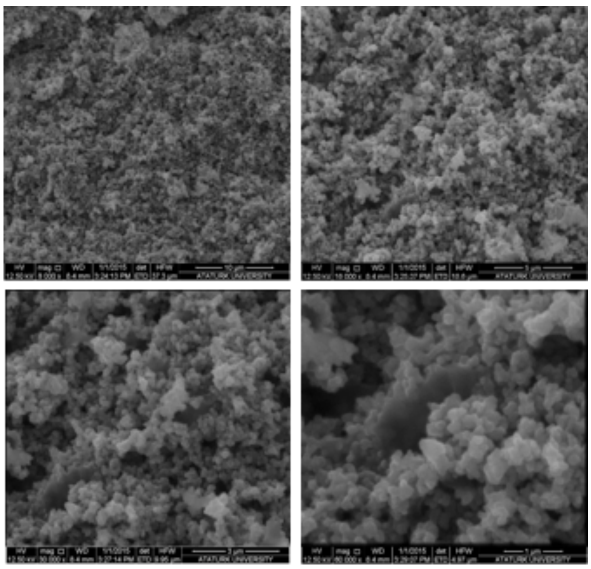

Figure 2. SEM images of biologically synthesized TiO_2 nanoparticles from precursor: titanium(IV) oxide.

EDX analysis was performed to determine the elemental composition and stereochemistry of the synthesized TiO_2 nanoparticles. As it is seen in Figure 3 and Table 1, EDX analysis results also confirmed that "Ti" and "O" are the basic units of these nanoparticles.

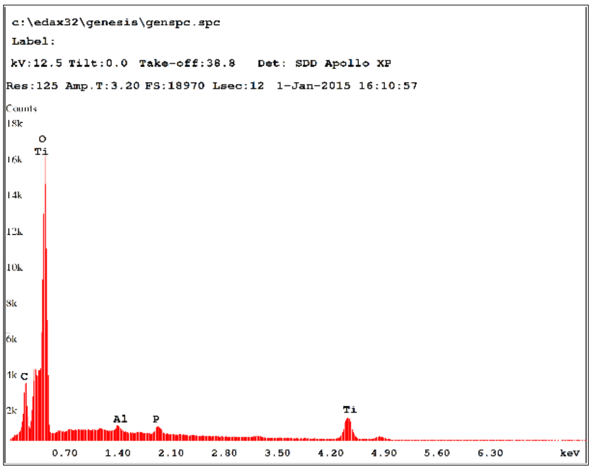

Figure 3. EDX results of biologically synthesized TiO_2 nanoparticles from precursor: titanium(IV) oxide

In the current study, TiO_2 nanoparticles were biosynthesized from titanium(IV) oxide precursor by *Aspergillus* sp. TK4 and their characteristics were investigated. TiO_2 nanoparticles were successfully acquired at the end of the biotechnological process. The results obtained compatible with the data in the literature that explains biosynthesis of TiO_2 nanoparticles from titanium(IV) oxide by *Aspergillus* sp. TK4 [17]. Furthermore, the occurrence of clustered structures besides TiO_2 nanoparticles may be explained by the assemblage of several nanoparticles to form a stable, non-uniform secondary size particle. This phenomenon arises from a large specific surface area and high surface energy level of nanoparticles and results in the formation of more stable aggregates and secondary microstructures [18].

Table 1. Percentage of elements present in the prepared samples

Element	Wt %	At %
C K	10.68	15.91
O K	67.21	75.15
Al K	1.04	0.69
P K	1.85	1.07
Ti K	19.21	7.18
Total	**100.00**	**100.00**

Recent studies showed that TiO_2 nanoparticles could be synthesized using various biological sources such as bacteria, fungi, and plant extracts [19]. For example, Santhoshkumar et al. [2] reported that TiO_2 nanoparticles was synthesized using *Psidium guajava* extract and revealed that these nanoparticles have antibacterial and antioxidant activity. Kirthi et al. [20] also synthesized nanoparticles using the bacterium, *B. subtilis*. Similarly, Bansal et al. [21] showed that TiO_2 nanoparticles could also be synthesized via fungi. With the optimization work to be done, the synthesis process can be standardized.

4. Conclusions

Given the literature, there are few examples of the use of fungi in the synthesis of TiO_2 nanoparticles. Unlike the studies previously carried out, extracellular enzymes produced by fungi were used to perform the biosynthesis process in our study. It has been shown that extracellular enzymes may act effectively in this biotechnological process. For future studies, it has been shown once again that the use of biological sources with unique properties in biotechnological processes has a significant place. The findings provide a source for new technologies to be produced in the future.

Conflict of Interest

The authors declare no conflict of interest.

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