Antibacterial Activity of Biosynthesized of Titanium Dioxide Nanoparticles Against Bacterial Contamination in Wastewater

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Abstract

In this study biosynthesis of titanium dioxide via aqueous orange peel extract was investigated. The prepared nanoparticles were characterized by different technique which include FTIR to provide information about functional group, XRD to give structure was anatase phase for Tio2NPs, average size of crystal was (10.7) (13.1) (18) nm of sample A, B, C of TiO2NPs respectively. FESEM to provide average particles size was 35.85, 46.44 ,56.64 nm and shape of nanoparticles was spherical for sample A, B, C of TiO2NPs respectively. EDX which give elemental composition of nanoparticles which confirm the presence of Ti element The antibacterial activity was investigated and confirmed by (colony forming unit). Tio2NPs causing 100% mortality on E-coli in all studied concentration and have activity on total count of bacteria.

Keywords: Titanium dioxide nanoparticles, Biosynthesis,Antibacterial activity

1. Introduction

Plant-mediated nanoparticle synthesis has recently attracted a lot of attention due to its inherent advantages such as speed, simplicity, environmental friendliness, and lower costs [1]. Nanotechnology is a branch of nanoscience that deals with synthesis and control of matter at the nanoscale[2]. Nanoparticles are particles with a diameter of less than 100 nanometres and a mass of 20–15000 atoms. Nanotechnology has recently gained popularity as a discipline with applications in optics, electronics, health sciences…etc. Nanoparticles are essential components of nanotechnology [3] When compared to bigger particles of the bulk material they are formed of, nanoparticles exhibit wholly new or 100nm, enhanced properties based on specific features such as size, distribution, and shape. With decreasing nanoparticle size, the surface to volume ratio of nanoparticles increases.TiO2 is a solid inorganic material, white metal oxide that is weakly soluble, nonflammable, thermally stable, and not classified as hazardous by the UN's Globally Harmonized System for Chemical Classification and Labeling (GHS) [4] Due to its vast range of applications, It became a commercial product for the first time in 1923. TiO2 NPs have received a lot of interest because of their amazing and favorable features in a variety of disciplines, including, improving healthcare, environmental concerns, biomedical devices, and drug delivery systems [5]TiO2 With a broad bandgap of 3.2 eV for anatase and 3.0 eV for the rutile phase, NPs are a well-known semiconductor [6]. There are numerous names for titanium dioxide, including Ti (IV) oxide, Titania, Titanic Acid Anhydride, and Ti White.[7] Tio2, is widely employed in cosmetics, photocatalysts, pharmaceuticals, sensors, and solar cell applications. The most common tree fruit produced worldwide is the sweet orange (Citrus sinensis (L.)). It is the best source of Vitamin C and is beneficial to both health and skin care [8]. It has a light yellow to orange peel that provides total protection for the fruit's inner section. Because it contains Citric acid as its principal source, orange peel functions as a reducing agent for TiO2 production[9].

2. Experimental

2.1 Preparation of orange peels extract

Orange fruit was bought at a supermarket in Kerbalaa City and washed multiple times with distilled water to remove any dust particles before being cut into little pieces. A 50 g orange peel was placed straight into the beaker and extracted for 2 hours at 90 ° c with 150 ml of water. What man filter paper was used to filter the extract. The filtrate was saved for future nanoparticle production [10].

2.2 Steps of biosynthesis of Tio2 include:

1-ultrasonically disperse (0.5, 1, 1.5) N titanium dioxide in 100 mL ethanol for 30 minutes was named as sample A,b,C respectively .

2-Added a drop of orange peel extract with wise, stirring constantly until the pH of the solution reached 7

3-At room temperature, the mixture was constantly stirred for 3 hours. In this procedure, nanoparticles are formed; these nanoparticles are separated using what man filter paper, and materials are repeatedly washed with distilled water to remove by-products.

4-Wet nanoparticles are obtained and dried overnight at 80°C

5-Finally, the particles are calcined for 3 hours at 600°C.[11]

2.3. collection of wastewaters

The study included taking wastewater in glass

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container from al-maamerah wastewater treatment plant at treated sewage outlet from Hilla city in March 2022 and transport as soon as possible to laboratory for culturing.

2.4. Antibacterial activity tests

Plate count method was used in this study. typical media include nutrient agar for general count and macconkey agar to count gram negative bacteria. Wastewater collected from sewage and cultured before and after treatment by different concentration of (0.5,1,2,5) mg/l as a powder for each sample of TiO2NPs. According to[12]the removal efficiency was determined by counting the number of colony forming unit and was defined as N = N1- N2/ N1 x 100 % where N1 and N2 , the number of colonies before and after treatment.

3. Result and Discussion

3.1. FTIR: is a simple analytical technique used to identify functional groups in solid nanoparticles. The absorption peaks appearing at 3402.75 cm-1 and 1650.76 cm-1 are due to the stretching and bending vibrations of the (O-H) bonds, which confirmed the presence of water molecules on the surface of the prepared samples. The weak absorption peaks at 2918.42, 2357.52, and 1132.56 cm-1 were assigned to N-H, C=O, and C-O bonds, respectively which are due to the carbohydrate and protein. The characteristic peak shown at 667.34 cm-1 is due to the stretching vibration of the (Ti-O-Ti, Ti-O) bonds. The results obtained are similar to previous studies prepared by [13]and[14].

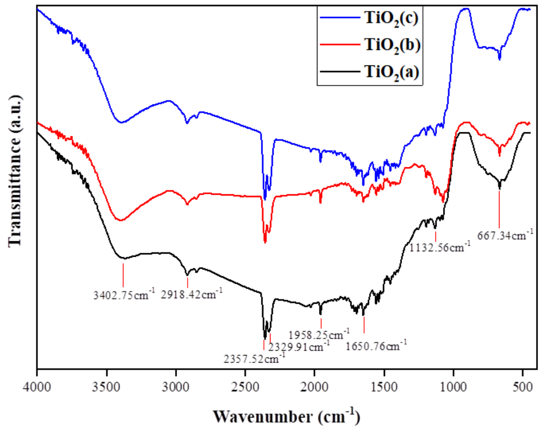

Figure .1. FTIR spectra for TiO2NPs

3.2.X-ray diffraction analysis: it is observed that the diffraction peaks of TiO2 NPs appear at 2θ =25.10, 27.20, 37.60, 47.70, 54.00, 62.30, 68.70, and 74.70 assigned to the hkl planes (101), (110), (004), (200), (211), (204), (116), and (215), respectively. It was found that the obtained diffraction peak positions are identical to the anatase phase diffraction positions according to the JCPDS files (NO. 21-1272). The crystal size of the TiO2NPs nanoparticles was calculated by Debye-Scherrer equation: D=kλ/βcosθ [15] it was found that the average crystal size of sample A,B,C are 10.7,13.1, and 18nm, respectively.

Table .1. crystalline data of different sample of TiO2

Sample	2θ	d (nm)	I %	FWHM	Crystal size (nm)	Average size
Sample A	25.11	0.354	100	0.691	11	10.7
	27.23	0.327	30.8	0.6	13	
	37.62	0.238	26.8	0.697	12	
	47.79	0.190	39.7	0.864	10	
	54.03	0.169	55	0.972	9	
	62.37	0.148	30.3	0.802	11	
	68.78	0.136	20.6	0.76	12	
	74.79	0.126	20	1.153	8	
Sample B	24.93	0.356	100	0.641	12	13.1
	28.25	0.315	16.9	0.697	11	
	37.44	0.239	39.6	1.579	5	
	47.84	0.189	43.4	0.625	14	
	53.74	0.170	25.3	0.559	16	
	62.55	0.148	32.8	0.781	12	
	66.50	0.140	11	0.544	17	
	68.49	0.136	14.7	0.791	12	
	70.20	0.133	11.3	0.481	20	
	74.85	0.126	19.9	0.822	12	
Sample C	25.04	0.355	100	0.369	22	18
	37.55	0.239	38	0.584	14	
	47.83	0.189	45.4	0.386	23	
	53.69	0.170	30.6	0.459	19	
	54.86	0.167	26.9	0.447	20	
	62.43	0.148	28.6	0.686	13	
	68.55	0.136	10.9	0.406	24	
	70.16	0.134	15.3	0.639	15	
	74.89	0.126	23.6	0.829	12	

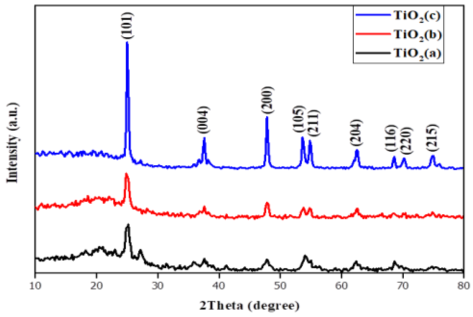

Figure.2. XRD patterns of TiO2 at different concentrations

3.3.FESEM: The results showed the presence of spherical shapes with different particles size and irregular distribution.in addition show the aggolermation of NPs due to orange peel extract which act reducing agent [16] [17]. The average nanoparticles size of the TiO2 samples (A, B, C) was 35.85, 46.44, and 56.64nm respectively.

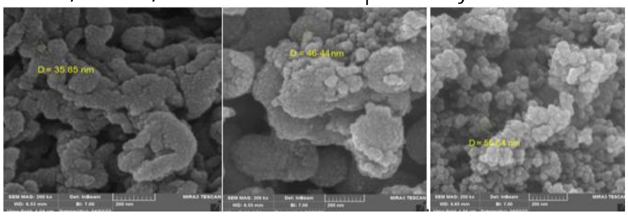

Figure .3. FESEM of different sample of TiO2NPs

3.4. Energy dispersive X-ray analysis (EDX): is an easy and common technique used to reveal the identity and quantity of the constituent elements of the prepared nanomaterials. The quantitative results confirmed the presence of titanium and oxygen elements with weight ratio are 57.22% and 36.90% for sample A. this indicates the success of biosynthesis TiO2NPs. Also, the presence of carbon, oxygen, phosphorous and sulfur elements due to the components of the orange peel extract used as a reducing agent in the preparation of TiO2 nanoparticles. The EDX pattern of sample B shows an increase in the intensity of titanium and oxygen peaks in weight ratios 61.79 and 27.63 % and as a result of the increase in concentration of titanium tetra isopropoxide during the synthesis process. The EDX pattern of (sample c) indicates the increase in weight ratio of titanium (87.19 %) and the ratio of oxygen (4.43). [18]

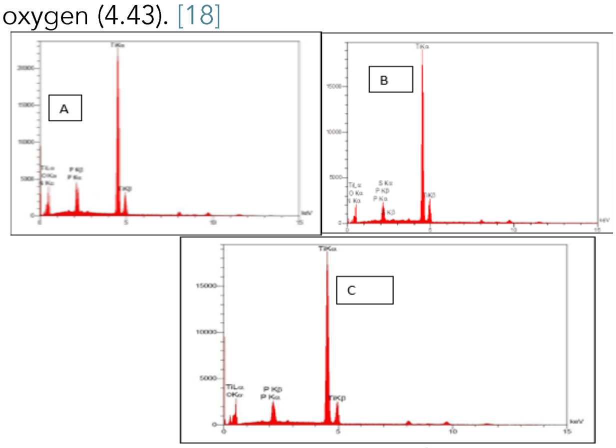

Figure .4. The EDX pattern of TiO_2NPs

3.5. Antibacterial

activity: Bacterial pollution of wastewater was tested against total count of bacteria and E –coli. before treatment with Tio2NPs total count of bacteria was 6100 CFU/ml and total count of E –coli was 4800 CFU/ml after treatment the number of bacterial colonies was decreased as in table 2.

Table 2: removal efficiency of different sample of TiO_2NPs .			
Concentration of nanoparticles	Removal efficiency of total count bacteria for sample A	Removal efficiency of total count bacteria of sample B	Removal efficiency of total count bacteria of sample C
0.5 mg/l	55 %	45 %	43 %
1 mg/l	67 %	50 %	45 %
2 mg/l	78 %	57 %	51 %
5 mg/l	87 %	63 %	62%

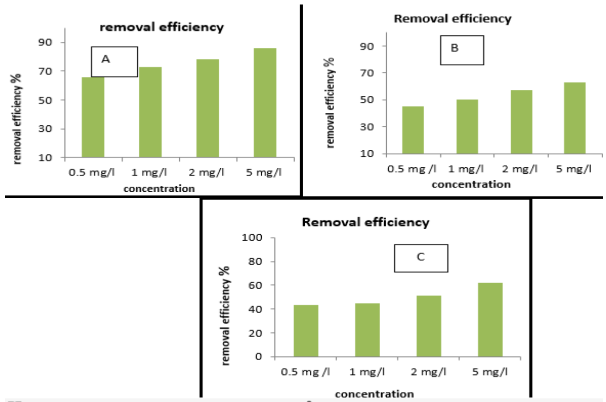

Figure .5. removal efficiency of different sample of TiO2NPs

With treatment ,Tio2 NPs was showed bacteriacidal effect against E-coli where causing mortality 100% in all studied concentration the result agreement with[19]. were showed biosynthesis of Tio2 with extraction of monsonia burkeana plant inhibited all colonies of E-coli. Bacteria are distinguished by their cell membrane, cell wall, and cytoplasm. Through direct contact between nanoparticles and cell surfaces, TiO2NPs penetrate bacterial cells. They easily penetrate through the peptidoglycan, making them extremely sensitive which effect on the permeability of membranes. The oxidative stress caused by the nanoparticles enable them to enter bacterial cells, where it inhibits cell development and ultimately leads to cell death. When compared to bulk particles, nanoparticles have a larger surface area. There is more area for interactions because of the higher surface area,lead to improve the bactericidal effect [20]. the mechanism of nanoparticles penetrates bacteria still investigated. The shape of the membrane alters as bacteria interact with metal oxide nanoparticles. This causes the permeability to rise, which interferes with normal transport across the plasma membrane [21], [22].Because of this, the bacterial cells are unable to control transit via the plasma membrane. As result the cells will die [23].Nanoparticles of titanium dioxide will penetrate inside bacteria [24]. After being exposed to titanium dioxide nanoparticles, cellular proteins will become inactive. Proteins and metal oxide nanoparticles interact, and they bind with protein molecules ,This inhibits the cellular metabolism and causes cell death [25]. In both the oxidized and reduced states, electron transport is increased by titanium dioxide nanoparticles [26] [27]. In general, bacteria have a negative charge while nanoparticles have a positive charge. The interaction between metal oxides, which have a positive charge, and the negatively charged bacteria results in the death of the microorganisms. As a result, there is electromagnetic interaction between the microbes and the metal oxides, which promotes oxidation and ultimately results in microbial death.[28]. in general, smaller nanoparticles offer more antibacterial activity Due to their ability to expose the bacterial membrane to more surface area.

4. Conclusion

Green synthesis of metal oxide nanoparticles by plant extract is novel approach in field of nanoscience and nanotechnology When used as a capping and reducing agent, plant extract reduces the metal ions, causing the formation of nanoparticles. Tio2NPs constitute effective antibacterial agent against pathogenic microorganism. Tio2NPs have bacteriacidal effect against E-coli.

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