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RESEARCH PAPER

A New Green Method for Synthesis of Manganese Oxide Nanoparticles as Antibacterial Agent Against Oral Biofilm

***Mohsen Safaei , and Ayoub Moghadam** **

Department of Materials Science and Engineering, Razi University, Kermanshah, Iran

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ABSTRACT

One of the most common human diseases is tooth decay, which is caused by several factors. Members of the group *Streptococcus mutans* have been identified as the leading causes of plaque formation and tooth decay. This examine aimed to optimize the inexperienced synthesis of manganese oxide nanoparticles through Bacillus sp. in opposition to *Streptococcus mutans* biofilm. For this purpose, nine experiments had been designed the usage of the Taguchi method. Meanwhile, the consequences of 3 factors, manganese acetate, glucose, and incubation time, had been investigated at 3 levels. The effects confirmed that the nanoparticles synthesized in experimental situations nine containing 1 mg/ml manganese acetate, eight mg/ml glucose, and seventy two h of incubation time had the best antibacterial interest in opposition to *Streptococcus mutans*. The characterization of synthesized nanoparticles become completed through UV-Visible spectroscopy, Fourier transforms infrared (FTIR) spectroscopy, X-ray diffraction (XRD), subject emission scanning electron microscopy (FESEM), X-ray power diffraction spectroscopy (EDX), and transmission electron microscopy (TEM). The effects acquired from the evaluation of checks performed, consisting of crystal shape and fuzzy identification, appearance, and length of nanoparticles, evaluation of chemical properties, showed the premiere situations for nanoparticle synthesis. The average size of nanoparticles synthesized using field scanning electron microscope image was determined to be 19 nm. This study showed that manganese oxide nanoparticles produced by the green synthesis method have optimal antibacterial effects against dental biofilms that cause tooth decay.

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INTRODUCTION

Poor oral hygiene, followed by bacterial accumulation and biofilm formation are the main causes of increased dental problems [1]. Oral *streptococci* are an crucial factor of dental plaque, and one of the maximum crucial participants of this series is *Streptococcus mutans*, which performs an crucial function in dental decay. This bacterium is a gram-positive coccus and optionally available anaerobic this is a part of the regular plant life of the mouth [2]. Using new technologies such as nanotechnology to make antimicrobial

* *Corresponding Author Email: moghadam.ayoub@gmail.com*

compounds to improve oral hygiene; can prevent the growth of bacteria, plaque formation, and tooth decay [3].

Various synthesis strategies for the manufacturing of nanoparticles had been proposed, together with physical, chemical, and organic strategies, the synthesis of nanoparticles. Conventional methods use toxic and harmful chemicals for the application of purifiers and reducing agents to prevent nanoparticles from agglomerating. Biological agents such as enzymes, plant extracts, and microorganisms are used to prevent the production of toxic and harmful substances; this method is called green synthesis [4]. At present, they have gained more attention because of the more attractive properties given by nanoparticles synthesized by the green method than by other methods [5]. Nanoparticles produced by the green synthesis method have higher stability, better and safer properties than other methods. Nanoparticles synthesized by physical and chemical methods typically have lower biocompatibility and stability and, under the same conditions, cannot be easily generated on a large scale [6]. Various microorganisms consisting of bacteria, fungi, algae may be used to synthesize nanoparticles from the aqueous answer in their metallic salts [7]. *Bacillus sp*. is a predominant soil microorganism, which can produce many antimicrobial compounds such as peptides, lipopeptides, and phospholipids. This bacterium may be used as a element for the synthesis of metallic nanoparticles [4].

Nanoparticles are broadly utilized in numerous fields because of their properties [8, 9]. Transfer metallic oxides had been taken into consideration because of their properties, inclusive of electrical, magnetic, and optical properties, skinny d-layer, and multifaceted nature of metallic ions. These oxides have high stability and have improved magnetic, optical, and thermal properties. For the element manganese, the transition metal oxides differ in layer d3. This gives rise to structural types such as MnO_2, Mn_2O_3, Mn_5O_8, MnO, and Mn_3O_4 [10, 11]. MnO_2 is one of the most important manganese oxides that has been considered by many researchers due to its electromagnetic properties. MnO_2 has great physical and chemical properties and is one of the stable manganese oxides. MnO_2 nanoparticles are used in ion exchange, superconductors, catalysts, energy storage, molecular absorption, biosensors, and drug delivery [12]. The production of manganese oxide nanoparticles has economic advantages and is compatible with the environment. Therefore, because of the low toxicity of those nanoparticles, they may be produced on a massive scale for biological uses. Manganese oxide nanoparticles have many structures that have different physical and chemical properties. Various nanostructures are made of manganese oxide [13, 14].

The use of the Taguchi method has an effective role in the correct and accurate design of tests, improving the results, and reducing costs. The Taguchi method is based on the partial factorial method. In this method, several possible combinations are selected between variables, and this selection is such that the variance of the error is the same as when all possible combinations are executed. In this way, by reducing the number of tests, their execution time is greatly reduced and causes saving time and costs. For this objective, in this research, the Taguchi technique was examined to determine the ideal circumstances for the eco-friendly production of manganese oxide nanoparticles by Bacillus sp. with the greatest antimicrobial effectiveness against biofilm *Streptococcus mutans*.

MATERIALS AND METHODS

Biological production of manganese oxide nanoparticles

Nine trials were planned using the Taguchi approach to identify the most favorable circumstances for the production of manganese oxide nanoparticles by bacteria. The bacterial strain of *Bacillus sp*. IBRC-M 11083 obtained from the Iranian Biological Resource Center. After preparing an isolated colony from the bacterium, to prepare the cell mass, it was incubated in a combined culture medium using different amounts of glucose (2, 5, and 8 mg/ml) as a carbon source at 30 °C and 160 °C in a shaker incubator for 48 min. The obtained bacterial masses were centrifuged at 6000 rpm for 15 min to separate the supernatant containing reducing agents (sugars and proteins). Then 50 ml of a solution containing concentrations of 0.1, 0.5, and 1 mg/ml manganese acetate were added to 250 ml flasks containing 50 ml of supernatant solution. The resulting solutions were incubated at 30 °C at 160 rpm for 48, 72, and 96 h. The ultimate solutions containing nanoparticles were initially filtered through Whatman filter paper to eliminate impurities and subsequently

sterilized utilizing a 0.22 micron filter [14].

Antibacterial activity of MnO_2 nanopowders

Streptococcus mutans PTCC 1683 (ATCC 35668) had been prepared from the gathering middle of Iranian Research Organization for Science and Technology (IROST) to analyze the antibacterial activity of manganese oxide nanoparticles synthesized via way of means of the inexperienced method. They had been cultured in mind coronary heart infusion agar media for twenty-four h to put together a unmarried colony. Then bacterial suspension equal to 0.five McFarland turned into organized. The bacterial suspension turned into introduced to a 96-properly subculture plate and incubated beneathneath cardio situations for seventy two h at 37 °C to shape a bacterial biofilm. The subculture medium turned into modified each day with fresh brain heart infusion containing 2% sucrose and 1% mannose. After biofilm formation, it changed into washed 3 times with PBS to eliminate planktonic *Streptococcus mutans*. The produced nanoparticles were subsequently introduced into each cavity based on 9 experiments devised by the Taguchi technique, and the dish was left to culture for 24 hours. The cells extracted from the cavity lining were gathered following 24 hours of incubation at 37 °C to quantify the quantity of viable cells in the biofilms. The leftover cells sticking to the cavity wall were diluted in three ml of PBS solution after three rinses. The resulting suspension was subsequently mixed using a vortex for 2 min. The bacterial suspensions were diluted 10-fold using serial dilution, then each dilution was cultivated on plates containing brain heart infusion agar and was kept at 37 °C for 24 hours to conduct the colony formation unit (CFU) examination. Following incubation, the quantity of colonies was tallied, and their mean was calculated for 9 trials. All trials were replicated three times [15, 16].

Characterization of manganese oxide nanoparticles

The UV spectrum of manganese oxide nanoparticles was recorded the usage of a visible-ultraviolet spectrometer of the Thermo withinside the variety among 2 hundred and 800 nm. Structural or chemical evaluation was done via way of means of Fourier rework infrared (FTIR) spectroscopy with a tool made via way of means of Thermo business enterprise, the Avatar model. Crystal shape and fuzzy identity have been completed via way of means of X-ray diffraction (XRD) take a look at with a tool made via way of means of Philips business enterprise, PW1730 model. The look and length of the nanoparticles have been tested via way of means of a subject emission scanning electron microscope (FESEM) with a tool made via way of means of a Tescan business enterprise and a CM120 transmission electron microscope made via way of means of Philips business enterprise.

Tauc equation, $(\alpha h\upsilon)^{\gamma}=A(h\upsilon-E_g)$, was used to calculate the optical band gap of nanoparticles from Uv-Vis absorption peak. In this equation, h is Planck's constant, α is the absorption coefficient, υ is the incident frequency, A is a proportionality constant and E_g is the bandgap energy. The γ factor depends on the nature of the electron transition and is equal to 1/2 or 2 for the direct and indirect

Table 1. The experimentation process using the Taguchi technique and the impact of artificially created nanoparticles on the biofilm of *Streptococcus mutans*, specifically in terms of their ability to combat bacteria

Experiment	Manganese acetate (mg/ml)			Glucose (mg/ml)			Incubation time (h)			Bacterial survival (Log10 CFU/ml)
	0.1	0.5	1	2	5	8	48	72	96	
1		0.1			2			48		5.17
2		0.1			5			72		3.82
3		0.1			8			96		4.04
4		0.5			2			72		1.82
5		0.5			5			96		1.51
6		0.5			8			48		2.39
7		1			2			96		1.43
8		1			5			48		1.68
9		1			8			72		1.21

transition band gaps, respectively.

RESULTS AND DISCUSSION

Antibacterial activity

The consequences of nanoparticles synthesized in distinctive situations at the survival rate of *Streptococcus mutans* micro organism have been evaluated in nine experiments to decide the foremost situations for the synthesis of manganese oxide nanoparticles with the very best antibacterial activity primarily based totally at the Taguchi method (Table 1). The results confirmed that the synthesized nanoparticles with situations of one mg/ml manganese acetate, eight mg/ml glucose, and seventy two h incubation time (test nine), had the most powerful antibacterial interest towards the *Streptococcus mutans* micro organism's biofilm. In this condition, the bacterial survival rate became the lowest at 1.21 CFU/ml. Previous studies have also shown that nanoparticles and nanocomposites synthesized by the green method have optimal antibacterial properties [17-22]. The application of MnO_2 nanoparticles to the cell resulted in alterations in the cell's structure, through which the nanoparticles effectively entered the cell, leading to harm to the cell's outer layer and subsequent release of cellular materials (Fig. 1).

Table 2 indicates the impact of manganese acetate, glucose, and incubation time at the survival rate of Streptococcus mutans bacteria. The results confirmed that the manganese acetate component has the best overall performance at the third level. Also, glucose elements and incubation time at degree 2 had the best impact at the survival rate of Streptococcus mutans bacteria.

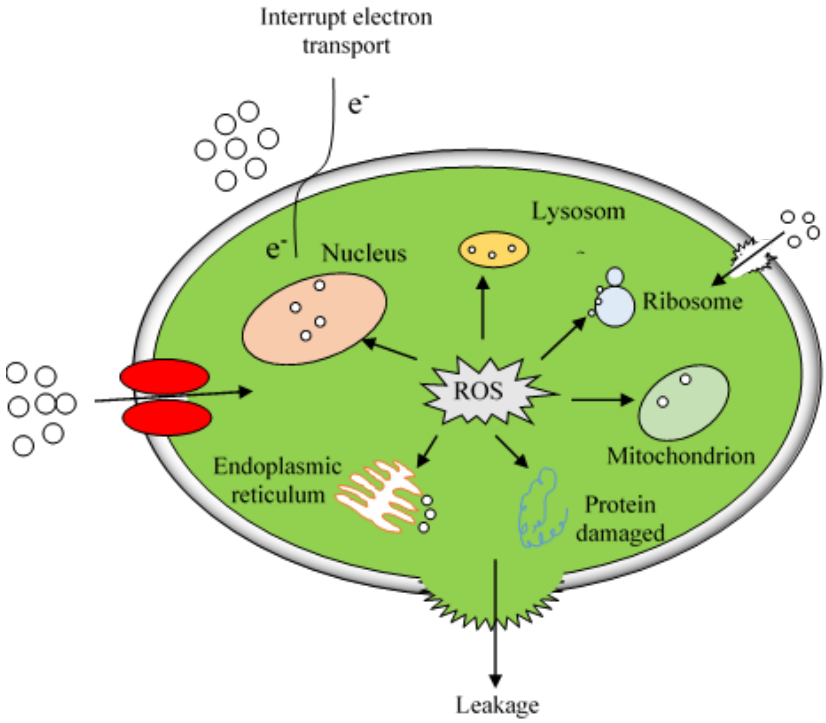


Fig. 1. Antibacterial mechanism of manganese oxide nanoparticles.

Table 2. Main results of various levels of manganese acetate, glucose, and incubation time on increase inhibition of *Streptococcus mutans* biofilm

Factors	Level 1	Level 2	Level 3
Manganese acetate	4.34	1.91	1.44
Glucose	2.81	2.34	2.55
Incubation time	3.08	2.28	2.33

The correlation between the examined variables on the viability percentage of Streptococcus mutans microorganisms is illustrated in Table 3. Glucose at the tertiary level and duration of incubation at the secondary level exhibited the most significant interaction and impacted the viability of Streptococcus mutans microorganisms with a value of 69.31. Manganese acetate at the third tier and glucose at the third tier exhibited a noteworthy interplay on the viability percentage of Streptococcus mutans microbes with a value of 13.13. The minimal intensity index of the interplay was associated with manganese acetate at the third tier and the duration of incubation at the second tier was 9.84%.

Table 4 presents the variance analysis of the factors influencing the viability of Streptococcus mutans microorganisms. The most significant impact on the survival rate of Streptococcus mutans bacteria was observed with manganese acetate, accounting for 90.21% of the variation. The incubation time and glucose had effects of 7.32% and 1.93%, respectively.

After studying the information and analyzing the effect of every factor, the optimum conditions for the synthesis of manganese oxide nanoparticles with the very best antibacterial activity had been estimated (Table 5). Accordingly, manganese acetate had the best impact, and glucose had the least effect at the survival rate of *Streptococcus mutans* bacteria. Incubation time had an effect among those elements and close to the incubation time.

Although the third tier was identified as the most appropriate tier for manganese acetate, the second tier proved to be suitable for both

Table 3. Interaction effects of the studied parameters at the increase inhibition of *Streptococcus mutans* biofilm

Interacting factor pairs	Column	Severity Index (%)	Optimum conditions
Glucose × Incubation time	2×3	69.31	[3,2]
Manganese acetate × Glucose	1×2	13.13	[3,3]
Manganese acetate × Incubation time	1×3	9.84	[3,2]

Table 4. The assessment of variance of factors impacting the growth suppression of *Streptococcus mutans* biofilm

Factors	DOF [1]	Sum of Squares	Variance	F-Ratio (F)	Pure Sum	Percent (%)
Manganese acetate	2	14.58	7.29	673.12	14.56	90.21
Glucose	2	0.33	0.17	15.35	0.31	1.93
Incubation time	2	1.20	0.60	55.57	1.18	7.32

[1] DOF, degree of freedom.

Table 5. The prediction of optimal conditions for the synthesis of manganese oxide nanoparticles with the highest antibacterial activity

Factors	Level	Contribution
Manganese acetate	3	-1.12
Glucose	2	-0.23
Incubation time	2	-0.28
Total contribution from all factors		-1.63
Current grand average of performance		2.56
Bacterial survival at optimum condition		0.93

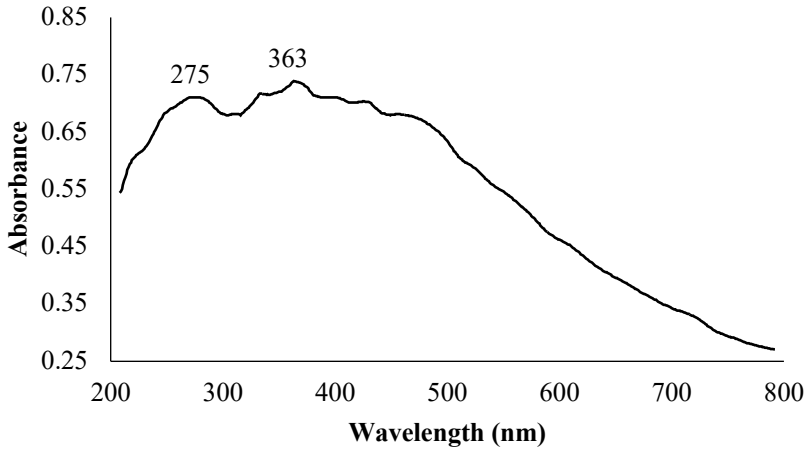


Fig. 2. The visible-ultraviolet spectroscopy of manganese oxide nanoparticles.

incubation time and glucose. Based on the findings, it was calculated that the nanoparticles produced under ideal circumstances could suppress the activity of *Streptococcus mutans* bacteria at a rate of 0.93 CFU/ml. This quantity is more optimal compared to the outcomes achieved in experiment 9, which demonstrated the highest level of effectiveness.

UV-vis analysis

The properties of manganese oxide nanoparticles had been investigated the usage of visible-ultraviolet spectroscopy withinside the variety of two hundred to eight hundred nm (Fig. 2). For manganese oxide nanoparticles, two absorption peaks in the absorption spectra were detected within the 275 and 363 nm range, indicating the distinct size distribution of manganese oxide nanoparticles. The optical band gap of nanoparticles, calculated from Tauc equation, is equal to 3.42 eV at the maximum absorption peak of 363 nm.

FTIR analysis

Fig. 3 displays the FTIR spectra of MnO_2 nanoparticles within the wavelength range of 400-4000 cm^{-1}. The FTIR spectrum of nanoparticles exhibited peaks at 3415, 2924, 1618, 1105, and 5484 cm^{-1}. The analysis of FTIR spectra for manganese oxide nanoparticles reveals the contribution of bacteria in the reduction process of nanoparticles. Peaks obtained in the

FTIR spectrum indicate that proteins, alcohol compounds, and cell wall components may be

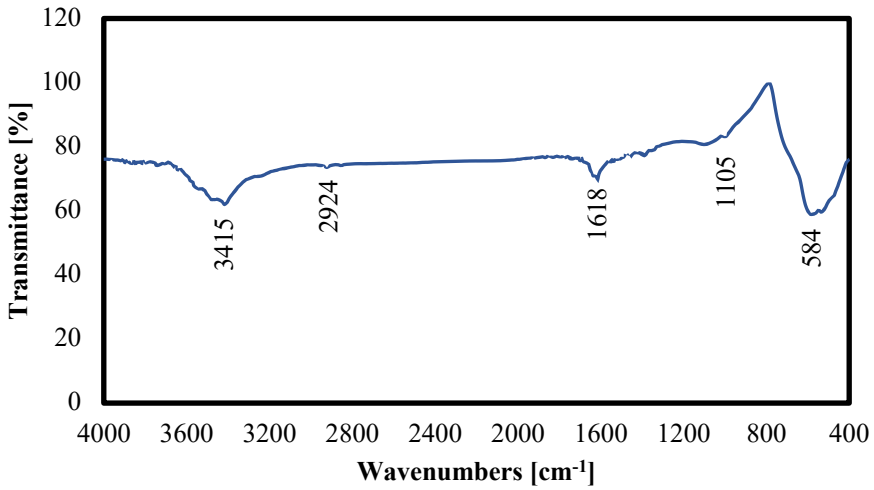


Fig. 3. The FTIR spectrum of manganese oxide nanoparticles.

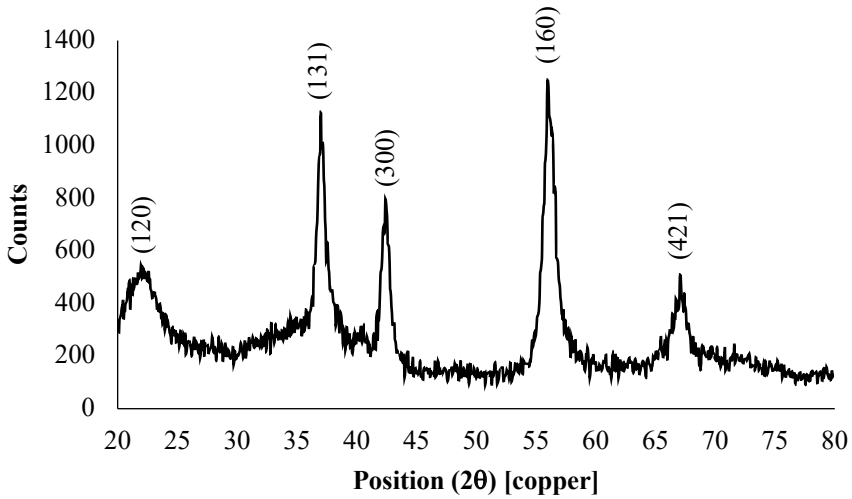
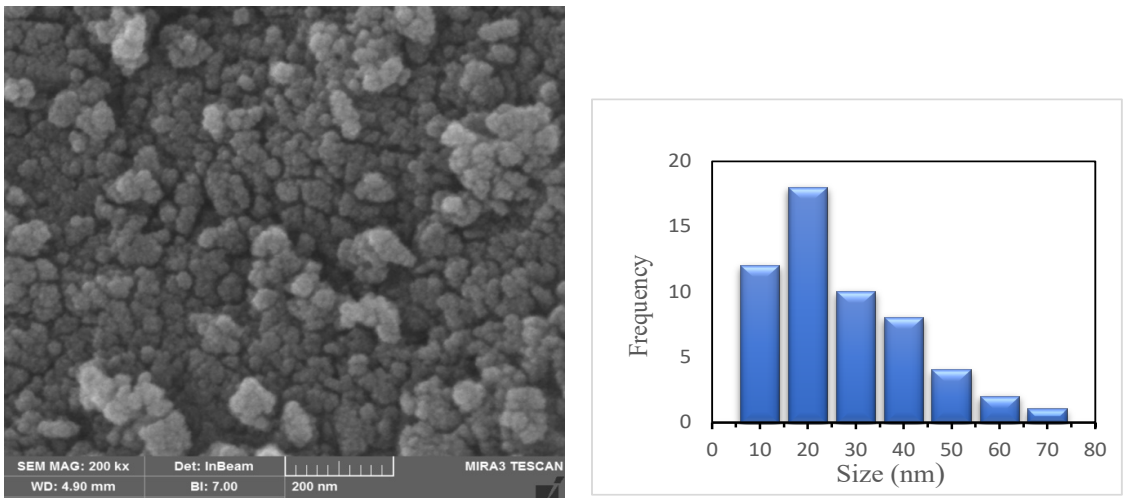


Fig. 4. The XRD X-ray diffraction spectroscopy pattern of manganese oxide nanomaterials.

involved in the synthesis and stabilization of manganese oxide nanoparticles. The extensive adsorption observed at wavelengths ranging from 3000 to 4000 cm^{-1} suggests the stretching interaction of H-O-H adsorption and hydroxyl. The crest in the range of 1618 cm^{-1} exhibits the spectrum of manganese oxide nanoparticles in the bending impact of the adsorbed water. The absorption crest at the wavelength near 1105 cm^{-1} reveals the surface clusters of OH from Mn-OH for manganese oxide nanoparticles. Mineral formations like MnO_2 possess more robust connections and less powerful oscillations that diminish maximum strengths in the FTIR diagrams. The assimilation maximum within the range of 400 to 800 cm^{-1} is connected to the O-Mn-O stretching repercussion. The FTIR graph in the current examination exhibited distinctive spikes at 584 cm^{-1}, validating the existence of MnO_2 manganese oxide nanoparticles [21].

XRD analysis

The phase formation and crystallographic results of manganese oxide nanoparticles synthesized the use of X-ray diffraction are provided in Fig. 4. The X-ray diffraction analysis pattern verifies the γ



(a) **(b)**

Fig. 6. The energy dispersive X-ray (EDX) pattern of manganese oxide nanoparticles.

Table 6. Elements of the sample of manganese oxide nanoparticles identified by energy dispersive X-ray (EDX)

Elt	Line	Int	Error	K	Kr	W%	A%
C	Ka	5	3.4662	0.02	0.0159	4.48	9.79
O	Ka	172.7	3.4662	0.3451	0.2749	38.29	62.85
Mn	Ka	221.3	0.9218	0.635	0.5058	57.23	27.35

phase with the hexagonal arrangement of MnO_2. The average crystal size

for the tallest summit was computed utilizing Debbie Scherer's equation, where D is the mean crystal size, λ is the X-ray wavelength (1.5556 Å), θ is the Bragg diffraction angle, and B is the full width of half the maximum which was measured using the Gaussian curve at the peak of (160). The average crystal size of manganese oxide was determined to be 17.62 nm. The dispersed peaks withinside the diffraction pattern of manganese oxide nanoparticles at angles 2θ identical to 22.5 for plane (120), 37.06 for plane (131), 42.48 for plane (300), 55.98 for a plane (160), and 67.19 for the plane (421) were observed, respectively [22, 23].

SEM analysis

Fig. 5 examines the morphology and length of synthesized manganese oxide nanoparticles with the aid of using field emission scanning electron microscopy. According to the field emission scanning electron microscope image, it can be concluded that the approximate size of most of the synthesized nanoparticles is in the range of less than 20 nm. The particle size distribution histogram diagram also confirms this (Fig. 5b). The SEM picture shows that most nanoparticles are spherical and in large part agglomerated. This is because of the small length of the nanoparticles and the sharp growth of their particular surface place because of the undesirable cold sintering of the nanoparticles.

EDX analysis

Table 6 and Fig. 6 show the constituent elements of manganese oxide nanoparticles identified by X-ray diffraction spectroscopy. The EDX spectrum of manganese oxide nanoparticles consisted of manganese with a mass of 57.23%, oxygen with a mass of 38.29%, and carbon with a mass of 4.48%. The existence of a small amount of carbon was identified as a contaminant that may have originated from the remaining organic constituents

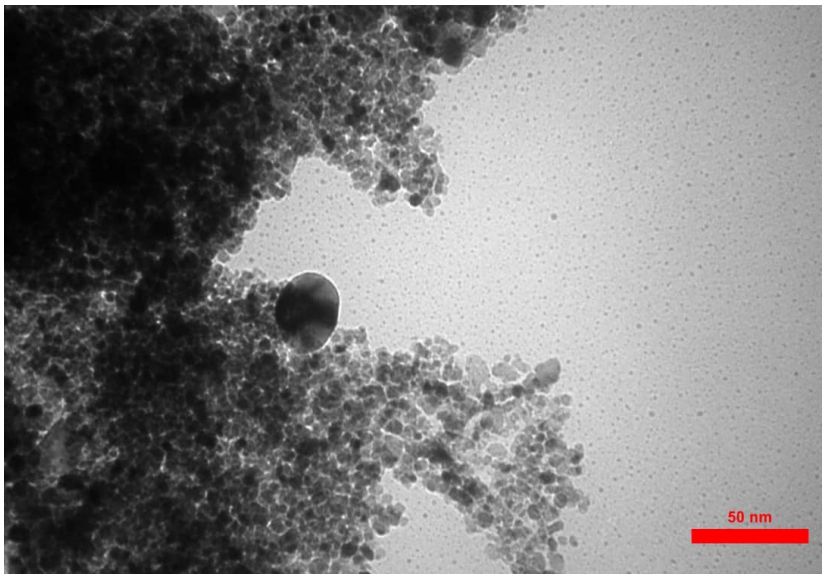


Fig. 7. The transmitted electron microscope image of manganese oxide nanoparticles.

employed in the creation of nanoparticles.

TEM analysis

The TEM image of the manganese oxide nanoparticles produced under ideal circumstances is displayed in Fig. 7. The image depicts nanoparticles that are both appropriate in size and nearly spherical in shape. Manganese oxide nanoparticles with a size less than 20 nm are created, and their ability to combat bacteria can be heightened by expanding their surface area. Prior research has also indicated that diminishing the size of nanoparticles enhances their effectiveness in fighting microbes [24-26].

CONCLUSIONS

In this research, *Bacillus sp.* became used to optimize the synthesis of manganese oxide nanoparticles with the best antibacterial activity making use of the Taguchi approach. Nanoparticles synthesized in a culture medium containing 1 mg/ml manganese acetate, eight mg/ml glucose, and seventy two h of incubation time (test 9) confirmed the best antibacterial activity towards the *streptococcus muntas* bacterial biofilm. The synthesized nanoparticles have been evaluated under optimal conditions the usage of UV, FTIR, XRD, FESEM, EDX and TEM tests. The effects of the analyses showed the synthesis of manganese oxide nanoparticles with suitable antibacterial homes and characteristics. The effects of this observe confirmed that manganese oxide nanoparticles synthesized via way of means of the green approach may be used as an powerful antibacterial agent towards dental biofilms.

This study stands out from previous similar research in several ways.

Firstly, the optimization of the synthesis of manganese oxide nanoparticles using the Taguchi method is a unique aspect of this study. This method allowed for the identification of the optimal combination of factors, such as manganese acetate concentration, glucose concentration, and incubation time, to achieve the highest antibacterial activity against the streptococcus mutans bacterial biofilm. The application of the Taguchi method in this context enhances the efficiency and effectiveness of the synthesis process.

Secondly, the use of Bacillus sp. as a means to optimize the synthesis process is notable. Bacillus sp. is known for its ability to produce bioactive compounds and has been utilized in various biotechnological applications. Its specific use in this study suggests a novel approach to nanoparticle synthesis and underscores the potential of utilizing microbial systems in nanomaterial production.

Furthermore, the comprehensive characterization techniques employed in this research, including UV, FTIR, XRD, FESEM, EDX, and TEM tests, provide a thorough assessment of the synthesized manganese oxide nanoparticles. The confirmation of suitable antibacterial properties and characteristics through these analyses adds credibility to the potential use of these nanoparticles as an effective antibacterial agent against dental biofilms.

Overall, this study introduces a unique approach to optimizing the synthesis of manganese oxide nanoparticles, explores the use of Bacillus sp. in this process, and provides in-depth characterization and evaluation of the synthesized nanoparticles. These factors distinguish it from previous studies and contribute to the advancement of knowledge in the field of antibacterial nanomaterials.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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